

nature

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The machinery works—does it produce the goods?

IN 1972 the British government published a White Paper 'Framework for Government Research and Development' which recommended re-structuring of financial support for R & D. The White Paper sprang largely from a report written by Lord Rothschild, and proposed, amongst other things, that support for applied R & D, whether conducted within or outside government departments should be controlled by the customer/contractor principle: departments as customers define their needs; contractors advise on the possibility of meeting these needs and undertake the work; and customer and contractor keep in close contact to ensure that objectives remain obtainable at a reasonable cost.

Of course, much departmental in-house R & D had always run along these general lines, but the controversial element of the Rothschild recommendations was that a substantial amount of work supported by Research Councils, particularly in their own establishments, should no longer be funded through the Science Vote (and thus ultimately by the Department of Education and Science) but be paid for by customer departments; research councils would continue to provide management for such activities, but the customers would control their direction. Customer departments were expected to acquire a Chief Scientist who would strengthen the department's ability to commission research widely. Chief Scientists would play an active role in research councils and also in the newly-formed Advisory Board for the Research Councils (ABRC) which took on a role of supervising the councils. The reorganisation could be seen as a corrective to a tendency for work in research council establishments to cover roughly the fields of interest of government departments without any obvious way for departments to express this interest and guide research, beyond gentle persuasion.

Has it worked? The Lord Privy Seal, quaintly entrusted with overseeing research and development policy co-ordination, has just issued, with a little help from his friends, a review of the R & D machinery as it now stands (Cmnd. 7499; £1.25). The answer, no great surprise, is that whilst there are still defects and weaknesses in the system, there is no call for further wholesale change in the arrangements.

The areas still needing attention, says the report, include the following. First, there is a danger that research councils (and, it could have been added, individual establishments such as the Institute of Geological Sciences) become so loaded with commissioned research that they lose independence to pursue their own ideas. The report sounds a particular warning over the Agricultural Research Council, which receives more than half its income from the Ministry of Agriculture, Fisheries and Food for contract work. Second, the report points out that the Department of Health has until recently had difficulties in fulfilling its role as informed customer in biomedical research, owing to the conflicting demands of health and social services research on the limited expertise available in the department. Third,

ABRC has, up to now, been too preoccupied with the Rothschild reorganisation and then with allocation of diminishing resources to devote enough time to "more general problems which arise from, or affect, the scientific activities of the government, research councils or universities". It is to be 'invited' to review its working methods to free more time for broader questions.

The machinery works tolerably well. But what of the product? It is not unreasonable to ask not only whether the right sort of bureaucratic connections are now being made, but also whether these are bearing fruit in terms of research results of real value. On this the report is silent—two paragraphs entitled 'The Dissemination and Utilisation of Results' comprise only generalities. Of course it is much more difficult to reach objective judgements on whether customers and the nation are really profiting from the new arrangements than it is just to verify that the arrangements meet general approval. Of course some of the timescales involved are too long for answers to be given yet. But without a dispassionate and rigorous assessment (and more than the department's point of view would have to be taken into account), it is impossible to be sure that all the time and effort actually bears fruit. The report claims to "try to assess whether the scientific activities financed by the government are now properly related to national need." It does not do this; someone should.

LESS publicised amongst Rothschild's recommendation was that scientists in government should be enabled to play a larger role in working out departmental needs and in policy formulation by improving their managerial skills. This was to be achieved by courses in management training, competitive transfer of scientists to the Administration Group and the stimulation of staff interchange with universities and industry. Here the report valuably notes that there has been miserable failure. Few civil service scientists are interested in jumping to administration and few are prepared to do even a short time outside the service—the inter-change unit was disbanded last year.

It is difficult to know what is wrong. Scientists are often to be heard grumbling that they don't get as many chances as administrators—"always on tap, never on top". Yet there are always apparently reasons why they never take the plunge. If they stay in science, however, many lose their enthusiasm and spend an unhappy ten or twenty years up to retirement.

Individuals have to recognise that no switch in career can be made without at least temporary inconvenience and struggle. Government has to recognise that private industry offers financial inducements to people it wants to move, and that it cannot expect much success without offering the same. A review of these manpower problems of the scientific civil service, under the chairmanship of Dr Martin Holdgate of the Department of the Environment, is just about to start to cover this ground yet again. □



Science exchanges at stake in SALT talks, says adviser

A FAILURE to agree on the terms of a strategic arms limitation treaty between the United States and the USSR could jeopardise the future of scientific exchanges between the two countries, Dr Frank Press, director of the Office of Science and Technology and science adviser to President Carter, said last week.

In a speech designed to encourage support in the scientific community for the second Strategic Arms Limitation Treaty (SALT 2), the terms of which are expected to be agreed shortly, Dr Press told a meeting organised by the State Department that if SALT 2 succeeds, other forms of cooperation—such as scientific exchange—could succeed. But if the agreement failed, it was difficult to see much chance for other kinds of cooperation to succeed.

"I am not saying we should sign SALT—any SALT agreement—just or even primarily to insure the future of scientific cooperation and exchange with the Soviet Union. What I am saying is that it is a clear side-benefit of SALT", he said.

Dr Press's remarks came as the administration is preparing a campaign to convince members of Congress,

some of whom have already expressed their opposition, to ratify the treaty once the terms are agreed by both sides.

Scientists had an important role to play in the debate on SALT, since before the Senate and the public could judge the treaty, they would have to understand it, he said. "Your views will carry special weight in the public mind on an issue like this. I urge you to play an active role and share your expertise and understanding of SALT with friends and colleagues."

An illustration of the type of issue involved in the forthcoming debate arose last week when Senator Henry Jackson announced that he was not going to support SALT 2, partly because he felt that the loss of US monitoring posts in Iran made the terms of the proposed treaty impossible to verify.

Earlier in the week, however, Mr Herbert Scoville, formerly deputy director responsible for scientific affairs in the Central Intelligence Agency, disputed this claim. He said that redundancy in the current monitoring system, as well as other verification systems such as satellites, was adequate

to ensure compliance, even with the loss of the Iran stations (much of the equipment from which is now being moved to Turkey).

Some scientists have expressed concern that, although they agree with Dr Press on the need to participate in wide public discussion of the technical aspects of the proposed SALT agreement, it will be difficult to do this adequately when much of the necessary information on, for example, monitoring techniques remains classified.

"I am quite prepared to encourage debate on the treaty provided that we can get the information on which such discussions can be based. But if we cannot get the necessary information, then I would be very doubtful about taking it up," Mr William Carey, executive officer of the American Association for the Advancement of Science, said last week.

Others are concerned that, in this eagerness to make the proposed treaty acceptable to a wide constituency in the Senate, President Carter is prepared to give the go-ahead to a range of new military technologies, thus doing little to curb the overall arms race.

David Dickson

Europe plans to predict earthquakes

EUROPE is to embark on a programme of earthquake prediction involving the collection of data on the ground and from satellites. A preliminary outline for the programme was drawn up by scientists attending a seminar organised by the European Space Agency and the Parliamentary Assembly of the Council of Europe in Strasbourg last week.

The programme is intended to monitor the Alpine-Mediterranean belt which runs from Turkey through Italy and Spain to the Azores. It is initially proposed to run from 1980-91 by which time a fully operational system of data-collecting stations should be in existence.

The possibility of an earthquake prediction programme for Europe was first raised last October at the Parliamentary Assembly of the Council of Europe by the chairman of the Assembly's Committee on Science and Technology, who comes from the Friuli region of Italy which suffered severe earthquakes in May 1976. He felt that because several of the member states of the Council of Europe are prone to earthquakes, efforts to predict them should be coordinated. Support from other parliamentarians was sufficient to warrant a detailed debate and the set-

ting up of last week's seminar.

The idea is to augment and co-ordinate earthquake prediction programmes already existing in individual states. The ground-based programme will involve collecting geophysical, geodesic, astronomical, geochemical and other data from 150 fixed stations and several mobile ones. Existing stations will be redefined to form part of a network and new ones will be built.

The space-based programme will involve imaging from satellites and aircraft, including laser-ranging and, later on, satellite-assisted very long base interferometry. Future geostationary satellites will be equipped with relevant imaging instruments. One of the aims is to draw a new seismic map of Europe.

The seminar decided that the European-Mediterranean Seismological Centre (EMSC) at Strasbourg should collect, analyse and interpret the data. It also recommended that a European Association for Earthquake Prediction be set up to run the programme, although initial responsibility will probably rest with the working party on geodynamics of the Parliamentary Assembly of the Council of Europe in conjunction with the European Space



Earthquake in Italy: a stimulus for the programme.

Agency, the European Seismology Commission and the Europe-Mediterranean Seismology Centre.

No estimate of the cost has yet been made. Rather than a pool of money being available for the programme as a whole, ground-based projects will probably be paid for by the state in whose territory they are and space-based projects will probably be built and paid for under ESA's new and optional Earth-oriented research programme.

Judy Redfearn

OTA faces another summer of uncertainty



David Dickson discusses the continuing troubles of the Office of Technology Assessment, whose third director in six years resigned earlier this month

LAST Thursday the twelve US Congressmen who make up the board of the Congress' Office of Technology Assessment met for the second time in eighteen months to discuss how to choose a new director for the agency.

The meeting was made necessary by the surprise resignation of OTA's current director Dr Russell Peterson (above), who took on the position last January. A chemist by training, Dr Peterson spent 26 years with the Du Pont Company, heading its research and development division until he was elected governor of the State of Delaware in 1968. In 1973 he was made chairman of the Council on Environmental Quality, which he left to set up a citizen's lobby group New Directions before moving to OTA.

Stating that his varied experiences in private and public life have "led me to prefer an advocacy role rather than an advisory one," Dr Peterson announced that he had accepted an invitation to become the president of the National Audubon Society, a body of 400,000 members whose main role is to protect the bird life of America.

In his 13-month occupancy at OTA, Dr Peterson has done much to put the agency on its feet, following a shaky start plagued by internal and external political wrangles. By streamlining day-to-day management, introducing clear hierarchies of responsibility, and other such moves, he has restored both OTA's reputation and its morale.

However it is no secret that Dr Peterson's strong views, both about how OTA should be run—he demanded, and got, complete authority to hire and fire staff—and on its role with respect to Congress, have themselves caused considerable friction (his resignation came not long after a particularly bitter board meeting in January).

Given that the aspects of Dr Peterson's personality which have exacerbated this friction are those that have helped place OTA on a more stable and effective footing, his departure has once again raised the

question of whether OTA—however desirable its objectives—can in fact function in the way that it was intended to do by Congress.

The Office of Technology Assessment was established by statute as an advisory arm of Congress in 1972. Its function is to help legislators anticipate and plan for the long-term consequences of technological applications, and to examine the many ways in which technology affects peoples' lives.

Few challenge the need for such analysis. And although the quality of the agency's reports have varied enormously, some—such as those on solar energy, on medical technology, and on a proposed coal slurry pipeline—have been widely praised, and played an important role in the development of legislation.

The big stumbling block has therefore not been whether, but how, such studies should be carried out. Under the first chairman, Mr Emilio Q Daddario—himself a former Congressman—OTA stayed close to the political process. This meant good contact with the political community; but the price was fierce political infighting that frequently spilled into the press, and consequently drained credibility from the agency's reports.

Under Dr Peterson's leadership the agency has, some feel, gone to the other extreme. By refusing to accept direct political pressure, he has eliminated many festering sores; but the criticism is that he has spent too much time telling Congress what it should be interested in, rather than listening to its demands.

Dr Peterson himself has strong, if controversial, views about technology assessment and its goals. "If we are to ensure change for the better, we must become skilled and successful architects of change. In today's interdependent world, that requires a holistic perspective—a comprehensive, world-wide, long-range view," he said.

The most obvious manifestation of this "holistic" approach has been an ambitious priority-setting exercise which

he set in motion last year. Over 5,000 people were asked to suggest critical technological issues facing the US and the world; to 1,530 topics suggested for study were added a further 2,875 extracted from the published literature. Synthesis and elimination by OTA staff led to a final list of 30 ranked items.

Few complained about the outcome. Of the final list, seven items were immediately selected by the board as areas for study, and three more subsequently added; these included alternative national energy futures, regulation and technological innovation, and the effects of nuclear war.

However a certain amount of resentment was caused by the fact that the exercise was initiated by Dr Peterson largely on his own initiative, and that by placing heavy emphasis on the exercise, he had shifted away from what they see as OTA's prime responsibility, namely responding to demands relating to specific legislation.

The issue of short-term versus long-term studies is endemic to discussions over the role of OTA, and is far from satisfactory resolution. And the need to maintain a balanced strategy places heavy responsibility on the shoulders of the director. The consensus in Washington seems to be that the new director needs the political skills and sensitivities of Daddario, the vision and managerial capabilities of Peterson, and a professional reputation respected in the scientific and technical community.

The task of finding a successor is therefore a daunting one. So much so that some are questioning whether OTA might not benefit from some structural change; for example, it could be more formally separated from political decisions by reducing the involvement of the 12 board members and increasing the independence of the director (a possible model might be Congress' General Accounting Office).

Many would welcome such a move, possibly with the idea of a scientific Brookings Institute in mind. Others, however, see it as a betrayal of the ideals that established OTA as a research tool forming part of, not a commentator on, the political process.

As usual in such cases, the last word may rest with those who hold the purse strings. Appropriations committees in both House and Senate are looking closely at OTA's budget request for 1980; possible cuts of \$2 to \$3 million (in a request of \$11.2 million), are being rumoured, and the final decision may well reflect Congress' reaction to the choice of a new director. It promises to be another long, hot summer for OTA. □

How the CIA backed research on mind control

David Dickson reports on recent revelations about the Central Intelligence Agency's support for university research

IN THE late 1950s and early 1960s, the US Central Intelligence Agency mounted a comprehensive and well-planned effort to encourage university scientists to carry out research that would, the agency hoped, lead directly to techniques for controlling the human mind.

According to previously classified documents, the agency was responsible for initiating or supporting research that led subsequently to important contributions in various fields of behavioural science, including sociolinguistics, psychotherapy and personality assessment.

And in an effort to legitimise its interest and obtain access to leading investigators, the agency financed a funding organisation that provided grants to a number of prominent members of the scientific community—including Carl Rogers, B. F. Skinner and Professor Hans Eysenck—even though the researchers were frequently unaware of the true source of their support.

The details of the CIA's involvement in these fields, described as helping to "liberate the behavioural sciences from the world of rats and cheese", are provided in a book which has just been published in the US by Mr John Marks, a former Senate aide and State Department official. (The book will appear in the UK later this year.)

Using documents obtained through the Freedom of Information Act, Mr Marks has put together a detailed picture of how the agency used the academic community in its search for the holy grail: a technique that would provide sufficient manipulation of the human mind to eliminate uncertainty from the intelligence business.

Many of the more bizarre, farcical or outrageous aspects of this pursuit have since received wide publicity. These range from experiments that switched the East coast academic community on to LSD, to the construction of a bugged San Francisco brothel (and included a scheme to make Fidel Castro's beard fall out).

But behind these there was, as Mr Marks shows, a closely considered plan for deriving, through the extension of conventional research techniques and the often unknowing cooperation of university scientists, the type of information that the CIA could use for controlling the behaviour of its agents and others.

Much of this research was financed through an organisation known as the Society for the Investigation of Human

Ecology, set up at Cornell University in 1955 under the guidance of Dr Harry Wolff, professor of neurology and psychiatry, who was later to become president of the American Neurological Association.

Dr Wolff's aim, which received considerable sympathy in various academic circles of the time, was to provide a framework for the multi-disciplinary study of man in relation to his environment. The CIA's interest was sufficient for it to provide 90% of the society's funding until, having separated from Cornell in 1957 to obtain "more established stature in the research community", the organisation was folded in the mid-1960s.

Some of the research funded through the society had a direct and obvious relation to the agency's interests. It provided Wolff himself with a research grant of \$74,000 to study the factors that cause men to defect from their countries and cooperate with a foreign government.

In other cases, the concerns were more basic. Professor Charles Osgood, for example, who had asked the CIA to support his research into the way people in different societies express similar feelings, received \$192,975 over five years through the society. He later completed his influential work on cross-cultural meaning on a grant from the National Institute of Mental Health.

In some cases, the CIA used research grants explicitly to build up the prestige of the Human Ecology Society. Thus the society provided \$26,000 to Professor Eysenck of London University to support his work on motivation; the agency accepted that the research would have "no immediate relevance to agency needs", but the society took funding credit for nine of his publications in its 1963 report.

"It's news to me", said Professor Eysenck. Often the research workers

were unaware of the source of the majority of the society's funds; some were told that the money came from rich New York doctors or Texas millionaires. "I don't like secret involvement of any kind. I can't see why it could not have been open and above board", says Professor B. F. Skinner, who received \$5,000 towards the research leading to his book *Freedom and Dignity*.

But as well as providing academic respectability, funding of research through the society allowed the CIA legitimately to approach “anyone in the academic community”, as one source put it. Thus in a move that Mr Marks describes as “turning scholars into spies”, the society provided \$15,000 towards a tour of the Soviet Union by ten prominent psychologists (only one of whom knew of the agency’s involvement), and CIA officials debriefed the group on its return.

Support for university behavioural research was curtailed in the mid-1960s. However, in contradiction of testimony later given to Senate committees, the research itself was continued, although with a greater emphasis on more direct ways of influencing behaviour, such as inserting electrodes in the brain. (At one point, agency scientists considered the potential of genetic manipulation: "The rest of the world didn't ask until 1976 the type of questions we were facing in 1965", one agency source is quoted as saying.)

Mr Marks admits that much of the research which the CIA sponsored led to important contributions to accepted academic knowledge—possibly because the agency was free to take imaginative leaps denied to more conservative (or more democratic) agencies. But he is uncompromising in criticising the ethical basis under which many of the experiments were carried out. (This was not always the agency's fault; some scientists had to be restrained from taking their experiments on, for example, sensory deprivation to their "terminal" conclusion.)

"We as a country can defend ourselves without sending our own scientists—mad or otherwise—into a hidden war that violates our basic ethical and constitutional principles. . . . The time has come for the United States to lead by example by voluntarily renouncing secret government behavioural research", Mr Marks writes in his conclusion. □



The Search for the 'Manchurian Candidate': The CIA and Mind Control by John Marks (Times Books, New York, \$9.95). The British edition will be published by Penguin.

UK atomic authority 'was a great mistake'—Benn

THE creation of the United Kingdom Atomic Energy Authority in 1955 "hived off policy formation as well as execution". It was, in retrospect, "a great mistake", the UK Secretary of State for Energy, Mr Tony Benn, said last week.

In a *Nature* interview with Mr Benn and his chief scientist, Sir Hermann Bondi, Mr Benn criticised the constitution of the UKAEA—under which research on waste management was relatively neglected for a long period. "If—in the creation of the Authority—the government had hived off Harwell and Aldermaston as research establishments, but kept the leading figures in nuclear policy within government, their relationship with the minister would have been very much closer than it is", he said.

Mr Benn has been minister in charge of the Authority from 1966 to 1970 and from 1975 to now. He believes "we have suffered" from its political autonomy.

In fact Sir John Hill, chairman of the United Kingdom Atomic Energy Authority, sought an interview with Mr Benn last week to discuss how to improve the flow of information between the Authority and the minister. After the meeting, Mr Benn announced that he would be sending a letter to Sir John outlining "a new list of areas of legitimate ministerial interest".

"It's not a question of control", said Mr Benn "it's a question of the integration of this very high technology with the democratic process. There are some very serious defects, but they don't arise from wickedness. They arise from the nature of the problem.

"Sir John came and said 'I tell you everything. What's wrong?'; but actually the problem of observing, watching, and connecting all these issues that a minister has, is quite different from the role of a manager of an authority. I am interested in all sorts of things—like, for example, what was that explosion in Russia in 1958? What happened to the 200 tonnes of uranium lost in the Mediterranean? I am interested in the movement of plutonium nitrates from Dounreay to Windscale, the real role of fusion, and all the areas relevant to the International Fuel Cycle Evaluation Programme. I try to preside over a range of issues as intelligently as I can, and need to keep informed on them all."

A second institutional problem, said Benn, is that in an agency set up to develop nuclear power the consequential effects of the technology "have tended to become subsidiaries to the agency's prime purpose". In practice,



Tony Benn: seeking more information on nuclear power

however, the effects have turned out to be very important.

"Where you fail to institutionalise basic conflicts that ought to exist in political discussion, then what happens is that the conflicts are solved at the Deputy Secretary level instead of at the Cabinet level. You must institutionalise the control of nuclear power to the point where all real arguments come to the top."

"The issues are enormous", said Mr Benn. He lists among them "the flow of information, the role of secrecy" in all high technology industries—including oil and gas—not just nuclear power. Also civil liberties, "where when you centralise, you then find the package deal includes a degree of intervention in civil liberties which is potentially unacceptable. All these things need to be brought out."

"In the end—however complicated a technology is—the public have the right in a democratic society to say no, as we've seen in Austria and very nearly saw in Switzerland. There is no escape from the ultimate provision of consent. The job of a minister is not to mystify, or to over-simplify, but to clarify, so that people know what they are doing. And you have to do it much earlier in the case of high technology."

"If it's a case of a road—do you want the M3 to go through Aldershot or not?—then you have a planning inquiry; but in the case of fast breeders, or nuclear power, or any other high technology, decisions will be taken that pre-empt things that will not happen for 10 or 20 years."

"The role of a minister as a public interrogator, who goes on asking questions, and then publishes the questions and invites the debate, is essential—otherwise you wake up one day and find that all the key decisions were taken 10 years ago. Then people get frustrated, they get violent, they get pessimistic, they get cynical. That is destructive of the whole democratic process, and it undermines the technology on which you have spent billions

and billions of pounds."

"This is my main interest in politics: how to get democratic control established effectively over areas where high skill, high complexity, high security, make the normal operations of democracy very very difficult."

Sir Hermann Bondi thought that the history of nuclear waste management "was an extremely interesting case". What the UKAEA had failed to foresee, said Sir Hermann, was not the need for the management of wastes, "but that there should be public anxiety about it".

In a sense the issue fell between two stools: "it fell into that gap between the institution and the politicians, so that neither saw that there would be an issue on which the public would become excited. The institution felt that all that the public were worried about was getting the power out of the plug cheaply, and they felt that technically the waste problem was soluble, so that there was no hurry yet to solve it."

Mr Benn illustrated the point with a story about cufflinks. "Have you had the British Nuclear fuel cufflinks?" he asked Sir Hermann. Sir Hermann had not. "I was sent a set of cufflinks by BNFL" said Benn "which is an ordinary set of cufflinks, but on the side where you would have a piece of jade or whatever, is a little brown, glass thing, and the card with them said 'These cufflinks represent how much vitrified waste you would generate through household usage in a year if all electricity were generated by nuclear power'. This was to show how small the problem is. They ignored the fact that they have got to last 20,000 years, and that even 40 million cufflinks make a pile." The idea that waste could be tackled by "the PR techniques of cufflinks" was "absolutely fantastic. I've never worn them because they are to me such a precious example of the wrong way of presenting a problem to people."

Robert Walgate

news in brief

SRC action puts new pressure on short-term contract workers: The Science Research Council has proposed a shift in funding emphasis from staff to equipment in a letter, written, "as a matter of urgency", to senior administrators at universities, colleges and polytechnics. Expressing concern about possible expenditure cuts arising from "the present industrial troubles", the council considers that the best way it can help research at present is to encourage applications for equipment. The council says "we cannot make additional major commitments to research grants which involve substantial expenditure over a period of several years such as those in which the provision of staff plays an important part". It urges speedy application before the next grant closing date of 1 April. [Brian Oakley, SRC secretary, says it is only partly a worry about the economic climate that has caused the shift in emphasis. "Good applications depend on good people and using the money for a three-year grant splurge will not bring in the young people that are necessary to swell the number of good applications."] Research workers on short-term contracts have expressed dismay at the SRC's action. "By spending money in this way, the SRC ignores the proven quality of senior researchers as shown by successive grant renewals through the highly competitive SRC grant selection process. Instead, the council is adopting a cynical policy of recruitment of younger workers who are cheaper and less knowledgeable about the system", says Robert Golub of the committee for long-term non-tenured faculty at Sussex University.



Levich appointed to New York chair: Professor Benjamin Levich (left), the Soviet dissident who was allowed to leave last year after a long campaign on his behalf by western scientists, has been appointed to the Albert Einstein chair in science at the City College of the City University of New York. Professor Levich has already become affiliated to Tel Aviv University in Israel. Dr Robert J. Kibbee, chancellor of City University, said last week that the appointment "helps to im-

plement an earlier agreement of cooperation reached between City University and Israeli universities." City College hopes to establish with Tel Aviv University a joint institute for applied chemical physics under Professor Levich's leadership.

Congolese geneticist still detained: This month marks the second anniversary of the arrest of Pascal Lissouba, agricultural geneticist and former Prime Minister of the Congo. Since his arrest and summary trial in March 1977, Professor Lissouba has been held under harsh conditions in the prison at Ouessou in the northern Congo. He is known to be seriously ill with kidney disease.

The Congolese Labour Party—theoretically the ruling body—is to hold its Third Extraordinary Congress later this month, and it is hoped that certain political detainees may be released in an amnesty for the occasion. The Congolese government, however, categorically denies having any political prisoners at all, and maintains that Professor Lissouba was involved in the assassination of President Nguabi in 1977.

Detailed enquiries by Amnesty International (whose charter precludes intervention in cases of political violence)

failed to find any substance in this charge—indeed, Professor Lissouba was adopted as an Amnesty International "Prisoner of the Month".

Professor Lissouba's arrest and imprisonment are almost certainly due to his political career during the 1960s. Although he had resigned the Premiership in 1966, after a disagreement with the then President, Massamba-Debat, and withdrew finally from politics in 1969, in order to devote himself to his work in cell biology, he was still, apparently, considered a focus of potential political opposition.

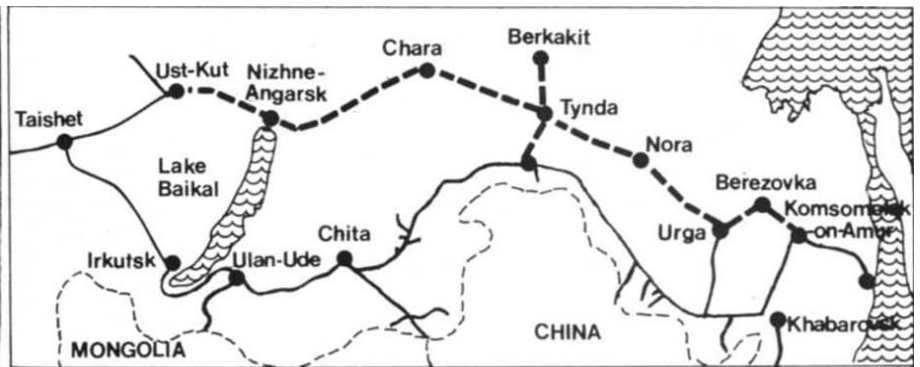
In 1976, when the military government was facing a general strike, Professor Lissouba was placed under house arrest for a time and deprived of his position as Professor of Cellular Biology at Brazzaville University. At the time of his arrest, he was under a restraining order forbidding him to leave the country.

Dow fights ban on 2, 4, 5-T: The Dow Chemical Company and 10 other plaintiffs are seeking an injunction to prevent the US Environmental Protection Agency enforcing its ban on the sale of the herbicides 2, 4, 5-T and Wilvex. They argue that the ban is based on a "seriously flawed study" which linked miscarriages in Oregon with herbicide spraying in nearby forests. They have asked a district court judge in Michigan, the chemical company's home state, to declare the EPA's emergency suspension orders "arbitrary and capricious", and to set them aside. The EPA has agreed to hold hearings requested by Dow and five other chemical manufacturers and users to consider lifting the emergency ban.

UKAEA responds to environmentalists' demands: The UK Atomic Energy Authority has agreed to let Friends of the Earth publish a critique of the Inhaber report on alternative energy in its magazine ATOM, subject to review by Professor F. R. Farmer, the AEA's health and safety adviser (1 March 1979). The AEA will also give FOE space to criticise Lord Rothschild's Dimpleby lecture on risk and will circulate a notice with its release of Rothschild's lecture saying that a contrary view is available.

California law on nuclear waste "unconstitutional": A federal judge in California has ruled that the state's law prohibiting the construction of nuclear power plants until a waste disposal system has been approved by the federal government is unconstitutional. The law has meant the virtual abandonment of plans for further nuclear power stations in California. Uncertainty over methods for disposing of nuclear waste has also led to similar moratoria in other states. Judge William B. Enright, however, ruled that the state law disrupted national plans for the development of nuclear energy, and that such preemption of the federal government was unconstitutional.

GMAG must cover industrial research, says the minister: Early fears that the widespread use of genetic engineering might be hazardous if organisms containing recombinant DNA were to escape "may have been mis-stated", Mrs Shirley Williams, UK Secretary of State for Education and Science told the House of Commons Select on Science and Technology last week. Nevertheless she does not think that this would justify moving "as far as NIH" in relaxing guidelines. In particular she feels that industrial research must come under GMAG's remit even though its inclusion means that some members must leave during discussions of a commercial nature. "If we said that no one could withdraw then we would have no industrial members or a lack of cooperation from industry".



Baikal railway raises environmental questions

"We are setting aside vast resources for the construction of the Baikal-Amur Mainline Railway" said Mr Kosygin, earlier this month. "Its commissioning will enable the rich natural resources of the trans-Baikal area, southern Yakutia, the Far East and Western Siberia to be more intensively exploited".

BAM—the Baikal-Amur Mainline—is due for completion in 1983. It has considerable symbolic importance to the Soviet planners. Its 3,000 km of through track (plus spur lines to major coal and ore deposits) will help to open up Siberia by providing a northern alternative to the existing Trans-Siberian route.

BAM was based on almost 13 years of surveying, before construction was begun in 1974—a survey which made extensive use of satellite photos, incidentally fulfilling Five Year Plan directives that the "exploration of near-Earth space" should be used "in the interests of the national economy".

Numerous research bodies were drawn into the planning of the project. A major role was played by the various institutes of the Siberian branch of the Soviet Academy of Sciences and the Far Eastern Science Centre. The Institute of Siberian and Far Eastern Geography has mapped the plant distribution of the BAM zone, classifying the various natural regions and determining the natural resources and the role of plants in erosion protection.

The same institute has also made a detailed survey of meteorological conditions, especially avalanches, in the

Kodar and Udokan ranges, while the Institute of Geology and Geophysics of the Siberian branch of the Academy has been working on tectonic movements. Preliminary results have been somewhat surprising. In the region of Lake Baikal, the rate of vertical movement of the crust appears to exceed that in the Caucasus, increasing northwards, so that Irkutsk is rising at 8 mm per year, and Nizhne-Angarsk (a station on BAM) at 19-20 mm.

Since the BAM zone is so highly seismic, the Institute of the Earth's Crust of the Siberian branch of the Academy developed a network of automatic seismic stations. So far, some 3,500 weak tremors have been registered and the system is shortly to be extended. Using aerial and satellite photography and computer simulation, a team from Alma-Ata are mapping sites of potential mud torrents in the future BAM industrial zone.

In addition to this seismic expertise, there have been several less expected spin-offs from BAM. One of the most interesting occurred on the section between the Baikal and Severomuisk tunnels, where the incursion of modern man turned up traces of primitive man, who, it would seem, first appeared in the area some 180,000 years ago.

Apart from problems directly connected with construction, however, the main direction of research has been ecology and conservation. From the beginning, BAM was planned with considerable respect for the environment. At times, the precautions seemed almost ludicrous, as when construction workers

were forbidden to use pesticides against the swarms of mosquitos which ruin the Siberian spring.

For the most part, though, the ecological planning was serious and well-founded. Round the northern shore of Lake Baikal, the original plan called for either a shore tunnel or else for the track to be laid along the actual shore of the lake. In the interests of conservation, however, it was finally decided to tunnel through the solid rock, to protect the lake from debris.

A vast nature reserve has been set aside in the BAM zone, including coniferous and mixed forest, mountains, rivers, lakes and alpine meadows. Fauna thus protected include wild elk, reindeer, black stork, white tailed sea-eagle, sable, brown bear, ermine and lynx. New legislation will ensure adequate crossing points for migrating animals to traverse BAM.

Planning is going forward, too, for the ecology of the future settlements of the area. Industrial complexes will be surrounded by green belts. According to Academician Brezhnev, Director of the Plant Breeding Institute of the All-Union Academy of Agricultural Sciences, "specialised farms" are to be set up along the route, which will produce fresh produce all the year round for the new towns. Sociological problems of life in remote settlements, and estimates of requirements in housing, education and recreation facilities are well under way.

However, perhaps the most unlikely spin-off from BAM is the tacit acceptance, in mass-media reports, that the seismic problems of the zone are due to plate tectonics. Until recently, Soviet geologists have been reluctant to commit themselves publicly to plates, due to the opposition of the prestigious Academician Belousov. Recent TASS releases, however, accept plate tectonics as an accepted feature of the BAM zone. Indeed, TASS reported last January that, owing to plate movements, by the end of the century, BAM will be some 50 cm longer than planned.

Vera Rich



The Baikal-Amur railway: route to the wealth of Siberia

Multiple aimpoints not the answer

The Carter administration is expected to decide next month which of a variety of possible basing modes it will adopt for the deployment of its new generation of intercontinental missiles, the MX. Richard Garwin here discusses how the alternatives relate to the current agreement on strategic arms limitation being negotiated with the USSR.

WIDELY heard around Washington these days is the acronym MAP, for Multiple Aimpoint System (also known as "MPS", for Multiple Protective Shelter). MAP has emerged as this year's leading contender to the endemic problem of the vulnerability of US intercontinental Minuteman missiles. But among the difficulties which MAP poses could be its impact on the structure and progress of SALT, the Strategic Arms Limitation Talks.

At present the United States deploys 1,000 Minuteman missiles in silos; a little over half contain three re-entry vehicles (RVs) per missile, the remainder only one. Minuteman silos are concentrated into six wings at separate locations in the United States. This concentration is in itself a weakness of the Minuteman force, for unless the United States chose to launch a planned strike it would be possible for the Soviet Union to create for a limited period an environment in which there was no point in launching missiles. Around one megaton detonated every minute above the atmosphere in the vicinity of each Minuteman wing would reliably 'pin-down' the missiles, which are fragile during the boost phase.

Such an attack, started perhaps with submarine launched missiles of short flight time and continued with land-based missiles, could not continue indefinitely. At some stage a direct attack would have to be made on the silos themselves. It is widely recognised now that Minuteman is not vulnerable to a short-duration attack unless enemy RVs are so accurate that no more than two (and preferably one) need be used against each silo. Even the low airburst required to produce sufficient overpressures on the ground to destroy a Minuteman silo throws a large amount of debris into the air, and causes problems with the penetration of a second RV to the target region.

The current interest in vulnerability arises from the improved accuracy demonstrated by Soviet missile tests. If the Soviet Union indeed deploys

2,600 or so RVs against Minuteman, with no systematic errors in targeting, and if the operational aspects of timing, command and control can be resolved, as well as the problem of retargeting backup missiles to replace those which have failed to emerge from the silo or have failed in boost, then the Soviet Union should be able to destroy a large fraction of the Minuteman force at some time in the 1980s—supposing Minuteman is not launched under attack or on warning of attack. Why it would want to spend 2,600 megaton-range RVs to destroy 2,000 submegaton-range RVs is a question which has not been given a compelling answer. The Soviet Union would reduce its own RV numbers (already below those of the US) by a larger fraction than would the United States, and would have expended three to four times as many megatons as it had destroyed. The US would still possess a large fraction of its 4,960 Poseidon warheads on nuclear submarines.

If the Soviet Union should choose to do this, however, MAP is an eminently rational proposal to counter Minuteman vulnerability. Grant the Soviet Union perfect reliability of its missiles and re-entry vehicles, essentially perfect accuracy and perfect knowledge of the locations of our land-based missiles, and the desire to destroy the US inter-continental ballistic missile force (ICBM). Grant that the US ICBMs would remain in their silos until after an attack, rather than be launched under attack or on warning. Then in principle one could preserve a substantial fraction of an ICBM force by siting individual missiles deceptively in a number of 'aimpoints' substantially larger than the number of RVs which the Soviet Union might bring to bear in an attack. A figure of 5,000–6,000 aimpoints has been discussed, and this could preserve more than half the US ICBMs against a Soviet force of 3,000 re-entry vehicles.

The number of US missiles actually secreted can be chosen to satisfy US force requirements, as can the size of the missile. One might imagine 1,000 single-RV missiles, each with an RV comparable with that on Minuteman III, or alternatively, a force of 200 large missiles in the same 6,000 shelters, each with 10 or 11 RVs of the same type. The choice of missile is strongly influenced by SALT considerations. The keystone of SALT II is equal numerical limits on ICBMs, and particularly on those containing multiple re-entry vehicles, and so the possibility of doing MAP under SALT leads one to consider very large mis-

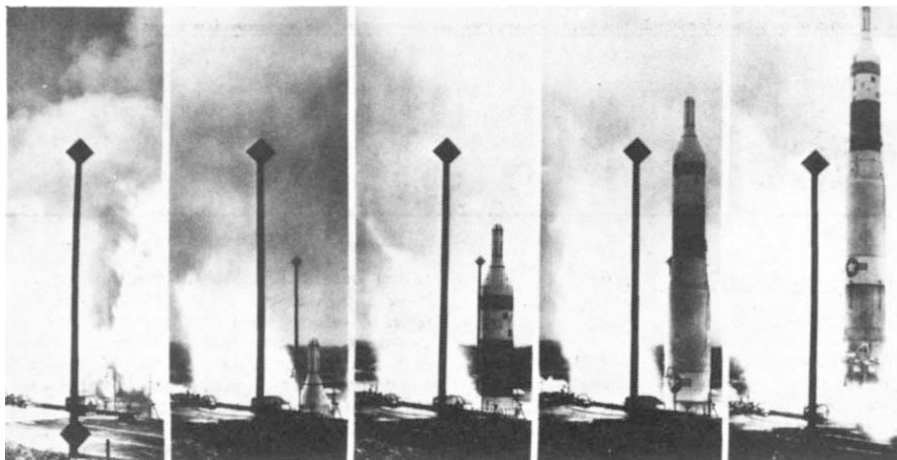
siles in order to have the largest throw-weight possible.

A typical breakdown of costs would be \$5 billion for missile R&D; \$3 billion for missile procurement (200 of 11 RV each); and \$20 billion for construction and maintenance of aimpoints. Because the cost of MAP is very largely in aimpoints, there is every incentive to reduce their cost—although that conflicts with the desire to have a large missile. On the other hand, a MAP-deployed ICBM force could be expanded greatly at little extra cost. With missile procurement a small fraction of total cost, there would be substantial interest in tripling or quadrupling the total missile throw-weight after deployment. This would be hard to resist unless constrained either by policy or by SALT.

Invulnerability of MAP is only guaranteed if the Soviet RVs remain fewer than the number which MAP is designed to counter. Even with achievable accuracies and reliabilities, a Soviet force of two RVs per MAP shelter could destroy a very large fraction of missiles, and the projection of such capabilities might lead to an early Soviet conclusion that MAP will be as vulnerable against the evolving Soviet force as is Minuteman now.

The MAP system would be even more vulnerable if the Soviet Union could learn which MAP shelters contained real missiles. Present discussions largely focus on shelters which contain either real missiles or decoys which look very realistic. Operational personnel probably must themselves be kept in ignorance of which shelters contain real missiles. When a missile is returned for service, for instance, its 29 decoys would also be returned.

The real problems with MAP are twofold. First, I believe that by the time MAP can be fully deployed, about ten years from now, the Soviet Union will readily have accuracies on the order of 100 m, allowing RV yields to be as low as 50 kilotons for effective destruction of MAP shelters. Such accuracy would come about not simply by improvement of inertial guidance systems, but also by capitalising on the availability of satellites (or ground-based radio transmitters) which will provide 10 m positional accuracy in three dimensions world-wide every 0.1 seconds. Thus it would be very difficult to argue that the Soviet Union, bent upon imperilling the land-based ICBM force, could not threaten MAP at modest cost. Further, the Soviet Union could readily stay ahead of the deployment of additional aimpoints if these aimpoints were sufficiently costly to



Underground missile sites are always vulnerable; in-shore submarines could be the answer



accommodate large missiles—probably around \$2 million per aim-point.

A second problem concerns leaving room for MAP within SALT (as demanded by Chairman of the Joint Chiefs General David Jones and by Secretary of Defense Harold Brown), for it would permit the Soviet Union to do likewise. Some may worry about the number of missiles the Soviet Union has deployed in its (hypothetical) MAP fields; I think the problem is a different one. The Soviet Union could manufacture a large number of MAP missiles, keeping them in store until it wanted to abrogate SALT. It could then deploy them within weeks into hardened MAP shelters already in existence and provided with good command, control, and communications. If a durable, accurate, commandable ICBM force does indeed convey a political advantage—as many critics of SALT assert, and hardly anyone is willing to challenge—the Soviet Union could in this way have seized an advantage unavailable at present.

For these reasons, I personally believe that we should not allow in SALT II the possibility of deployment of MAP, and that the US should not go ahead with MAP under SALT.

One basing mode for ICBMs free of pin-down constraints, and not vulnerable to improved accuracy and further fractionation of the throw-weight of Soviet ICBMs, would be to carry 6,000-mile range missiles horizontally outside the hull of many small submarines deployed close to US shores. Each submarine might weigh 350 tons and carry some 350 tons of missiles. At least 400,000 square miles is available within 200-300 miles of US shores,

far too large an area to be barraged by Soviet nuclear weapons. The proximity to the shore allows efficient use of submarine time even if the maximum speed were only 5 knots, for which a 30-kW propulsion plant would serve.

Available in the nearer term is the air-launched ICBM. Large military cargo aircraft would be stationed routinely, one or two at a time, at sparsely-equipped bases in the Central US. Each would contain one or more modern ICBMs which are launched by being dragged out of the aircraft by parachute, and fired within seconds.

Survival of the aircraft would be assured by rapid takeoff in response to indications by infrared warning satellites of attack. The aircraft could also be launched if this warning system were disrupted or destroyed, flying to one of a large number of dispersed military or civilian bases within the United States. Such a system could be made more secure if it were based on advanced short-take-off aircraft.

Because the missile would be launched from aircraft at altitude and speed, a substantial increase in number of RVs would be available in comparison with surface or submarine launch. Compared to conventional bombers, the military cargo aircraft would be much simpler and would have a higher readiness rate, thereby making the system cheaper for a given number of alert missiles. Unlike MAP, the system would preserve a very large fraction of the deployed missiles against the assumed threat. In the past, such concepts have encountered cost difficulties; it remains to be seen whether the management techniques used by the US Air Force in holding down

operational cost for silo-based systems can be applied to a new-generation ICBM and its aircraft.

Finally, because Minuteman silos are so numerous and considerably 'harder' than an RV they could in fact be defended at an affordable cost. This is not true of unique targets such as command centres, nor targets such as cities against which dozens of nuclear warheads could be used. Effective nuclear defence of Minuteman silos could be achieved by burying a nuclear explosive about 1 km north of each silo and detonating it under the control of an upward-looking mini-radar a few km further north. A non-nuclear defence which is receiving some attention uses similar mini-radars north of each silo to predict the arrival time and point of each RV at an imaginary screen some 1 km north of a Minuteman silo. At this point the RV would be met by a flight of 10,000-40,000 mini-rockets (about 500 g each) flying at a speed of 1,000 m/sec from a launching silo near the Minuteman silo. Because the radars look at the threat RVs from the side and not from the nose, and particularly because they see them at 2 km distance rather than 100-1,000 km range, they can be very small and cheap. Although they are more vulnerable to attack than a Minuteman silo when operating, the radars can be made effectively immune by the use of additional radars in mini-silos to be put into service after the operating radars are destroyed. Such a system might serve to prolong the life of Minuteman silos during the awkward period from 1982, when the Defence Department assesses vulnerability to be likely, to the completion of full-scale deployment of some new ICBM basing mode in 1988.

In conclusion, I believe that MAP is a non-starter. It is far too expensive, far too late in remedying Minuteman vulnerability (if such be important), essentially incompatible with SALT, and burdened with environmental problems. While the possibility of defending existing Minuteman silos suffers from lack of appeal to advanced technologists, it deserves more and urgent attention. In my opinion, the basing of a new generation of encapsulated ICBMs on near-ocean submersibles is also very attractive, and it may have some appeal in the modernisation of the strategic nuclear force of the United Kingdom. The more familiar concept of air launched ICBM's, though perhaps more expensive than the mini-subs, may be available sooner as an effective counter to Minuteman vulnerability.

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correspondence

Smallpox research at Birmingham

SIR,—We took note of your editorial (11 January, page 75) concerning Professor Shooter's report and related aspects of the smallpox case in Birmingham.

It may seem surprising to your readership that, to date, various statements and opinions concerning this report have gone unchallenged by Professor Bedson's colleagues. We, who are members of the Department of Medical Microbiology, should like to record that this does not imply acquiescence or agreement with public statements concerning the report nor indeed, with the content, interpretation and conclusions of the report. The Shooter report is not yet public and there is pending a prosecution from the Health and Safety Executive; on this account, it seemed improper to make public comment.

We think, however, that in certain areas of your editorial the reader is entitled to some factual information. For example, with respect to Dr Mark Darlow's statement that many academics still believe that "what was good enough for Pasteur is good enough for me", we feel that most academics would wish to know which of their fellows supported this contention; and later, with respect to Dr Darlow's comment: "Bedson knew he was backing a lame horse and that it would stumble sooner or later. It stumbled sooner," we should like to enquire what evidence led Dr Darlow to this conclusion and, further, was his conclusion based on personal knowledge of Professor Bedson and his department or on his own interpretation of the report?

It does not seem to accord with the opinion of grant-giving bodies who were supporting the work, nor with that of his academic colleagues in the Department of Medical Microbiology. One minor point—your editorial properly refers to Dr Darlow by his correct professional designation; we feel disappointed that Dr Darlow did not see fit to accord the late Professor Bedson a similar courtesy.

Finally, your extensive editorial, which we felt to be a very reasonable assessment of the Shooter report is, *de facto*, based only on the findings of the report and must, of necessity, depend on the assumption that the report is a correct and reasonable account. In our opinion, it is not a correct and reasonable account, and quite simply is unfair to Professor Bedson and his professional reputation. We hope for the courtesy of your columns at a later date (when the Shooter report is public) to communicate to the academic readership our personal reservations and criticisms.

Yours faithfully,

G. R. B. SKINNER
A. BUCHAN

Department of Medical Microbiology,
University of Birmingham, UK.

Undergraduates more numerate?

SIR,—Joseph Schwartz' interesting article on numeracy in Britain (1 February, page 344) gives obvious cause for thought as to the basis on which one can make any statement concerning supposed changes in numeracy over the years. So far as university entrants are concerned one hears many subjective views expressed, mostly of a pessimistic kind, but seldom are any hard facts adduced in support of these views. In the absence of any long-term research study having been carried out over the last decade or two it is hard to reach any conclusions that would satisfy the stringent requirements of a research publication, but our own experiences with biological sciences students at Leicester may provide a pointer, at least so far as one identifiable group of 18-year-olds is concerned.

In common with many other schools of biological sciences we find it necessary to teach a limited degree of basic algebra and statistics to our entrants, most particularly to those (over half) who have not read A-level mathematics. We have taught such a course to our first years since the inception of the school in 1968 and at least since 1971 the format, content and method of assessment of the course (mostly by computer marked multiple choice tests with questions drawn from a largely unvarying data bank) have remained unchanged. The average A-level attainment of our entrants, and the proportion having A-level maths have also not changed significantly during this period.

Until some three years ago one could safely predict that some 10–15% of our entrants would be almost entirely unable to cope with even the simple course which we give. At the other end, the numbers gaining a very high mark would never exceed 20%, and was often much lower. Each of the last three years, however, has seen a clear improvement over any previous year, either in the numbers failing, the numbers gaining a distinction, or both.

Other than an improvement effected three years ago in the physical arrangements for our problem classes (which could be significant) those of us who lecture in this course are not aware of any change in our teaching technique in the last three years, and indeed with the rise in the proportion of entrants who have taken "modern maths" at school a certain communication problem is beginning to be evident.

These observations clearly cannot be taken as definitive evidence for a rise in numeracy amongst our entrants, even though my subjective judgement would be that our current entrants are much less afraid of numerical work than their predecessors of, say, seven or eight years ago.

They most certainly, however, do not lend any credence to the notion of a decline in numeracy in the group concerned, and in areas such as computing and numerical biology in which we give

rather detailed courses at Leicester, we have good cause for satisfaction with the level of interest and attainment found. It would be interesting to know if other institutions which have admitted students over a number of years and have assessed their numeracy in some way have had similar experiences.

Yours faithfully,

A. J. ROWE

Department of Biochemistry,
University of Leicester, UK

Scientists should press for Freedom of Information

SIR,—As a public member of the all-party Parliamentary Freedom of Information Campaign (FOIC), I should like to draw the attention of the scientific community to the important private member's bill of Mr Clement Freud MP, called an Official Information Bill, which has been given an unopposed second reading (*Hansard*, Vol 960, No 38, 19 January), and is now in its committee stage.

The main purpose of this measure is to repeal the notorious "catch all" Section 2 of the discredited Official Secrets Act of 1911, and to replace it by a form of Freedom of Information legislation. This latter is broadly in accordance with the obligation of the United Kingdom as a signatory to the European Convention on Human Rights and Fundamental Freedoms, and with especial reference to Article 10 (freedom of expression) of the Convention. Thus, the Council of Europe (Report No 4195) calls upon "the governments of member states which have not yet done so, to introduce a system of freedom of information, ie access to government files, comprising the right to seek and receive information from government agencies and departments, the right to inspect and correct personal files, the right to privacy and the right to rapid action before the courts in these matters."

On behalf of the FOIC, I therefore urge all members of the scientific community to write to their MPs to support the Official Information Bill during its next stages in Parliament; and to make representations through their unions to similar effect. (Freedom of Information legislation is official TUC policy.)

And finally, I appeal to those members of the scientific community living in countries which have already introduced Freedom of Information into statute law, to impress upon their British colleagues, in their various contacts with them, the importance of such legislation both for the advancement of science (and the public interest) in the United Kingdom and for the broader aims of effective international cooperation.

Yours faithfully,

STANLEY ALDERSON

Cambridge, UK.

news and views

LEAD is an extremely useful metal and men have mined and smelted it for over 8,000 years. The enormous demand for the metal in classical times produced widespread and generally fatal plumbism among the slaves and prisoners who usually mined it. Occupational plumbism was for centuries a serious menace among lead workers, potters and in lead pigment mills (see Dickens 'A Star in the East' In *The Uncommercial Traveller*); however the general public was not exempt from the ravages of this disease. Widespread and frequently fatal colic epidemics, usually caused by lead-adulterated wines ravaged many parts of Europe until the 19th century (see *TIBS* 2, 147; 1977).

Environmental lead poisoning of comparable severity is fortunately rare today (but see 'Thai village finds tragedy on a road paved with lead [slag]', *New York Times*, July 5; 1977), but the levels of lead in the environment as well as in the bodies of inhabitants of industrial countries have

Living with lead

from Josef Eisinger

been rising along with the exponential growth of lead production and are now one or more orders of magnitude greater than the 'natural' levels. This has evoked considerable concern among the public as well as governmental health authorities grappling with the task of defining safe limits for environmental lead exposure.

The most important biochemical interactions of inorganic lead are characterised by the ions' propensity for binding to sulphhydryl groups which leads to the inhibition of many different enzyme systems. As a result the early symptoms of chronic lead intoxication are variable and nonspecific and the

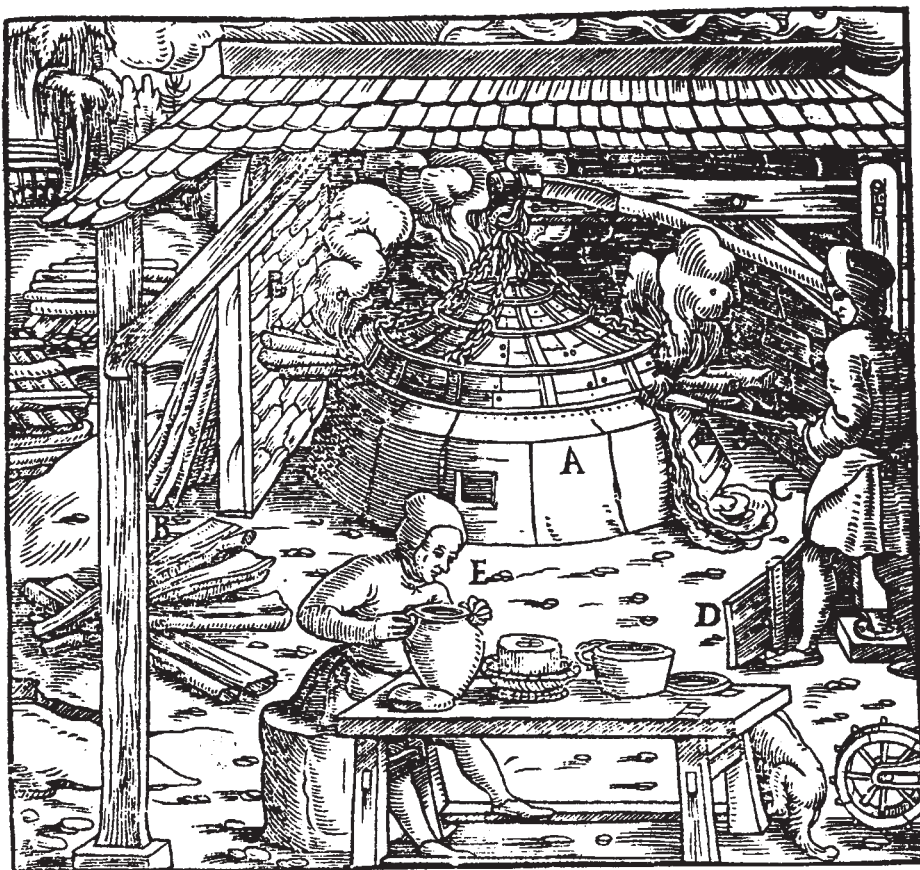
problem of relating exposure to disease (for example, effects on the nervous system) is a difficult one and is, because of its economic ramifications, often controversial.

A recent report (*Investigations into lead from motor vehicles*, Chamberlain *et al.*, Harwell, HMSO; 1978) illustrates one approach to improving our understanding. It presents the results of a painstaking experimental study of the pathways by which lead enters and leaves our bodies and what is the size and fate of the fraction which stays behind.

Since the study, which employs the tracer ^{203}Pb ($T_{1/2}=52$ h) as well as stable lead, is primarily concerned with emission from motor vehicles it includes ambient measurements of the concentrations and size distributions of lead aerosols near roadways. These are found to depend upon driving and weather conditions, with air lead levels near motorways ranging up to $10\text{ }\mu\text{g m}^{-3}$, about the same as in the vicinity of lead smelters. Air levels in busy London sites are broadly speaking about half of this value and inside a car the levels are only slightly lower. From the data given and some reasonable assumptions the amount of lead emitted along a road carrying an average of 1,000 cars per hour is $100\text{ g m}^{-1}\text{ d}^{-1}$, some 10% of which is deposited within 100 m of the road.

When such lead laden air is inhaled, approximately a third is deposited in the lung although this fraction is considerably higher for very small aerosol particles ($<0.05\text{ }\mu\text{m}$) and there appears to be a significant variation among the subjects who participated in these experiments. Clearance rates from the lung follow complex kinetics, depend on particle size and chemical composition and again show individual variability. About a day after a person is injected with or inhales ^{203}Pb , his blood lead level reaches a maximum and the total amount in his blood is approximately half of the dose given.

The efficiency with which lead compounds are taken up from the gut is also found to vary considerably from subject to subject (for example 2–26% for PbS) and depends on the chemical composition, with even nominally insoluble compounds (for example PbCrO_4) being taken up quite efficiently, presumably because of the high stomach acidity. A full stomach is found to offer a measure of protection:



This woodprint from Agricola's *De re metallica* (Basle, 1556) shows the smelter foreman eating from a tub of butter which, the caption explains, is a specific remedy against the poisonous lead fumes issuing from the smelter behind him.

A 12-hour fast increased the efficiency with which $PbCl_2$ is taken up from 7 to 45%. This appears to bear out the traditional advice offered to lead workers and potters by the earliest miners' physicians, to eat lots of fatty foods and soups (Stockhausen *Libellus de lithargyrii* . . . Goslar, 1656, see figure).

Chamberlain *et al.* (*op. cit.*) perform a great service by presenting a synthesis of their results and those of other investigators in the field. This should be useful in constructing kinetic models for the lead transport within the body. The factor relating blood levels (in $\mu g\ dl^{-1}$) to ambient air levels (in $\mu g\ m^{-3}$) for instance, is found to range from 0.5 to 2, depending on the investigator and/or the methodology employed.

The most significant conclusion from the point of view of environmental policy may be that at $1\ \mu g\ m^{-3}$, the average air level in British cities, the ratio of air lead to other lead intake is low, between 8 and 20%, and is in substantial agreement with the conclusion of another recent study (*Lead pollution in Birmingham*, HMSO, 1978). The importance of local conditions can be seen from the finding that the mean lead level in the blood of mothers and children in North Wales is doubled if their houses have lead, as opposed to copper piping (Elwood *et al.* *Lancet* **i**, 1363; 1978). This suggests that the greatest lead intake among the general population in Britain is caused by lead piping in soft-water areas.

A somewhat different approach to the problem of gauging the severity of environmental lead disease makes use of biological indicators of lead toxicity. While the blood lead level has been used traditionally for this purpose it suffers from serious drawbacks in that such assays are slow, expensive, require skilled personnel, have poor reproducibility and are prone to contamination errors. In view of the complex and variable kinetics of lead transport with-

in the body the blood level is moreover an indicator of uncertain validity for long term lead exposure. While elevated porphyrin levels have long been known to be associated with lead disease (Watson *et al.* *Pediatrics* **12**, 40; 1958) the zinc protoporphyrin (ZPP) level in blood has been demonstrated recently to be a superior indicator to blood lead, in that it was found to correlate better with the signs and symptoms of chronic lead disease and has, as will be seen below, numerous practical advantages as well. (Eisinger *et al.* *J. Env. Path. Tox.* **1**, 897; 1978; Grandjean *et al.* *Scand. J. clin. Lab. Invest.* **38**, 669; 1978).

The enzyme ferrochelatase inserts iron into protoporphyrin IX to form haem and is inhibited by lead ions at the site of erythropoiesis. When this occurs (or if there is a shortage of the substrate Fe^{2+} as in iron deficiency anaemia) zinc ions, which are available in the bone marrow are substituted for iron to form ZPP which is then incorporated in the globin (Lamola *et al.* *Science* **186**, 936; 1974). The resulting impairment of the blood's oxygen carrying ability is negligible since the haemoglobin of even seriously lead poisoned persons contains less than 1% of ZPP-globin. What makes this assay for lead toxicity so useful is that (1) ZPP levels in blood can be measured fluorometrically since ZPP fluoresces while the haem does not, and (2) ZPP-globin once formed, remains in the erythrocyte for the life time of the cell ($\sim 120\ d$) so that ZPP level in blood is an indicator with a 4-month integration time (Lamola *et al.* *J. Lab. clin. Med.* **56**, 1528; 1975). This is particularly important where exposure is chronic but intermittent.

In the US the ZPP assay has been recommended for the screening of children for lead exposure by the Center for Disease Control, with the test's response to iron deficiency anaemia being considered an additional advan-

tage. Recently the US Occupational Safety and Health Administration while retaining standard limits for environmental and blood lead levels, recommended ZPP assays for the medical surveillance of lead exposed workers (*Fed. Reg.* **43**, No. 220; 1978).

ZPP levels in blood have a considerable dynamic range. The blood of city and country dwellers typically register 50 and $20\ \mu g\ dl^{-1}$ respectively while that of lead workers can range up to $1,000\ \mu g\ dl^{-1}$ (Lillis *et al.* *Environ. Res.* **14**, 255; 1977). Central nervous system dysfunction, probably the most serious but elusive aspect of lead disease, has been shown to be significantly correlated with the blood ZPP levels (Valciukas *et al.* *Science* **201**, 465; 1978).

The ZPP assay is not only a meaningful indicator of lead disease but a particularly convenient one, because of the development of a portable instrument. This dedicated front face fluorometer, called a haematofluorometer, requires only a drop of blood, generally obtained by finger puncture (Blumberg *et al.* *J. Lab. clin. Med.* **89**, 712; 1977). The blood is smeared on a glass cover slip which is inserted in the instrument, and a digital display gives the ZPP level in convenient units within seconds. Since haematofluorometers have become commercially available, ZPP levels can now be measured quickly, accurately and at a small fraction of the cost of a blood lead determination. One may hope that ZPP assays will be useful not only for the screening of potentially exposed persons, but that they will make feasible large scale epidemiological studies of the general population to improve our understanding of the relationship between lead exposure and disease. □

Josef Eisinger, a scientist at Bell Laboratories, Murray Hill, New Jersey is at present a visiting Guggenheim Fellow at The Physiological Laboratory, Downing Street, Cambridge.

A hundred years ago

We are greatly disappointed and much surprised to learn that the application of the Scottish Meteorological Society for assistance to establish an observatory on Ben Nevis has been rejected both by the Meteorological Council, with its yearly 15,000*l.*, and the Government Grant Committee, with its 5,000*l.* The former had other matters to attend to, the latter handed the application over to the Council of the Royal Society, labelled "highly commendable." We have recently shown our readers with what heartiness and cosmopolitanism such nationally beneficial undertakings are managed in France; yet in this disgracefully wealthy country, with 15,000*l.* a year expressly devoted to meteorology, a plan of promoting meteorological research that would lead to results of the highest consequence must collapse for want of a paltry 500*l.* to start it. It would be a

shame if so really national an enterprise were to depend entirely on private subscription.
From *Nature* **19**, 13 March, 445; 1879.

A REMARKABLE phenomenon is reported from Neufchatel. On February 10 the Lake of Neufchatel suddenly assumed a motion like the sea with its tides, only with the difference that the rise and fall of the water succeeded each other in much shorter intervals. The phenomenon began at noon and lasted until 2 p.m. Boys who were playing on the shore were so suddenly surprised by the rising lake that they were up to their knees in water before they had time to escape. In the evenings there was a violent thunderstorm, which also visited Berne at the same time.
From *Nature* **19**, 13 March, 446; 1879.

INTERESTING correspondence has appeared in the British Guiana *Royal Gazette*, we learn from the *Colonies and*

India, relative to the qualities assigned to the fruit of the papau-tree. It has been recently asserted, in an article in the *Pharmaceutical Journal*, "that the most interesting property attributed to it is the power of its juice to render bad flesh tender." Mr. Monro, of Georgetown, furnishes certain facts which he says are commonly known to the natives of British Guiana relative to this fruit. A horse tied near one of these trees rapidly loses health, and a stud horse becomes useless. Any pressure on the body of the animal leaves an inelastic indentation. The sap of the tree will soften steel, and before the process of tempering was known in the Colony, the blacksmiths used to drive their brittle chisels and plane vices into the wood, leaving them there for a day or two; and tough meat wrapped in the leaf for only a few minutes becomes tender.

From *Nature* **19**, 13 March, 447; 1879.

Unified field theory: dream becoming reality?

from F. E. Close

EINSTEIN spent the latter part of his life attempting to find one formula that would explain both the gravitational and the electromagnetic field. Pauli believed this to be impossible stating "what God hath put asunder, no man shall ever join".

Einstein failed in his quest but recent theoretical developments in high energy physics, combined with sophisticated experiments, suggest that we may be at the threshold of a profound unification of electromagnetism with the weak force of radioactivity and also the strong nuclear force. This will leave gravity as the final link in a grand synthesis.

Thus the centenary of Einstein's birth is an appropriate moment to outline why the high energy physics community is so optimistic that such a grand scheme may be at hand, even though gravity itself is not yet included.

Electromagnetic and weak interactions

The electromagnetic force has been well understood for 30 years. It is mathematically described by quantum electrodynamics, QED, the theory of the interaction of charged particles with photons (the quanta of the electromagnetic field).

As long ago as 1934 Fermi had recognised some similarities between the weak β -decay of the neutron and electromagnetic phenomena. This led him to propose a model for the weak interactions. This model was perfectly adequate as a description of laboratory phenomena but was found to contain internal inconsistencies if applied to extremely high energies. In 1957 Schwinger showed that if there existed electrically charged W bosons then the deficiencies of the Fermi model could be made less serious.

These W bosons of the weak interactions have a role analogous to the photon in QED. They are predicted to be about 70 GeV in mass (the proton is but 1 GeV mass) and it will be another couple of years before machines will be developed that can produce them.

In the 1960s Glashow, Salam and Ward and also Weinberg were instrumental in using this idea to create a model which unified QED and the weak interaction. If the W bosons had been massless, like the photon, then 'weak' interactions and electromagnetic interactions would have had essentially the same strength and been manifestly recognised as being 'the same'. The fact that $W^\pm$ are so massive is the

reason why interactions with W bosons are so much weaker than those with photons and makes electromagnetism and the weak interaction seem to be different.

In 1970 't Hooft showed that this model was completely self consistent, violated no sacred principles and was thereby a candidate theory unifying the weak and electromagnetic interactions. Accumulating data during the past 5 years, and in particular in 1978 (see *News and Views* 264, 505; 273, 99; 274, 11; 275, 267) now give solid evidence showing that this theory is very near the truth, and could even be completely correct.

This theory is known as quantum flavour-dynamics, QFD, and contains QED within it. The unified electromagnetic and weak force is often referred to as the 'electroweak' force.

QCD: a theory of strong interactions

Particles like the proton which feel the strong nuclear force appear to be complicated systems of more fundamental particles called quarks. Quarks have electrical charge and flavour and so partake in interactions with photons and W bosons, that is, the electroweak interaction. They also appear to carry a further kind of charge known as a colour charge. It is hypothesised that there exist coloured gluons, analogous to the photon of QED, which couple to this colour charge and hence generate the strong interaction. The resulting theory is called quantum chromodynamics, QCD, and has many similarities to QED. This theory is still very young and its richness is only gradually being understood. Quantitative definitive tests are slowly being found and the first data that are being accumulated appear to be in excellent accord with the predictions (see *News and Views* 277, 349; 1979).

Grand unification

QED involves interaction of electrical charge with photons; QFD involves interaction of flavour with W bosons and QCD involves interaction of colour charge with gluons.

This qualitative similarity between the three theories is manifested mathematically, the three differing only in internal symmetry properties which are described by group theory. QED is a $U(1)$ field theory containing one 'gauge boson'—the photon. An $SU(N)$ 'non-Abelian' field theory has N^2-1 gauge bosons; hence three ($W^+W^-W^0$) for the $SU(2)$ QFD and eight gluons for the $SU(3)$ QCD (see for example *News and Views* 277, 349).

The idea behind grand unification is to find a super group G which contains $U(1) \times SU(2) \times SU(3)$ (QED, QFD and QCD). The simplest example for G is the group $SU(5)$. The above (N^2-1) rule shows that there will be 24 gauge bosons. Twelve of them will be the photon, three W bosons and eight gluons. A further dozen are thereby predicted.

The $SU(5)$ supergroup accommodates a pair of leptons (for example e^- and ν) partnered by a pair of quarks (u and d). The quarks necessarily have colour while leptons do not. These features are all in accord with experience. This pairing of a lepton doublet with a quark doublet has been recognised for some time and the set is often referred to as the 'first generation of elementary particles' (see *News and Views* 271, 406; 1978). Nature appears to have made xerox copies: a second generation ($\mu^- \nu'$ and cs) and possibly a third generation ($\tau^- \nu''$ and $t(?)b$). This is acceptable in $SU(5)$, if somewhat unnatural.

The twelve new gauge bosons of $SU(5)$ can cause quarks to transmute into leptons. This will make the proton unstable (see below) and may provide a test of this idea. Indeed this prediction of proton instability is not unique to the $SU(5)$ idea and occurs in other candidate schemes where G is the group $SO(10)$ or the exceptional group E_6 . These different groups make different predictions for the menu of quark and lepton flavours that await discovery at the new accelerators.

Asymptotic freedom and grand unification mass

The possibility that we may be able to invent a sensible grand unification scheme has come from the discovery of the electroweak unification and also the possible discovery of the laws governing the strong interaction (QCD). Of particular significance in the latter case has been the discovery of the property of 'asymptotic freedom'. This appears pivotal to the grand unification idea.

The strength of the strong interaction at low energies is much greater than the electroweak interaction. The quark-gluon coupling decreases in strength as energy or mass increases until asymptotically it tends to zero (this is 'asymptotic freedom'). There will therefore exist an energy scale where the 'strong' interactions are no longer strong, but have comparable strength to the electroweak. At such an energy 'unification' can have a well defined meaning.

This energy scale can be calculated, and turns out to be about 10^{19} GeV, and so in a realm where gravity may also begin to be important. In the original heat of the big bang such energies may have been common and a true unity may have existed. As the Universe cooled so the different interaction strengths froze out giving the strong, electromagnetic and weak interactions of the present day.

The original symmetry of the hot Universe has gone. The heat generated in high energy accelerators has managed to give a glimpse of nature soon after the big bang and reveal hints of that long lost unity. As energies are increased so one reaches nearer to that original symmetry. Therein is the aesthetic beauty of particle physics and the grand unification ideal.

Progress so far

At present, high energy physicists are concentrating on deepening their understanding of QCD and of trying to find the correct 'super group' that will enable it to be unified with the electroweak interactions.

The simplest candidate for this unifying group is SU(5), proposed by Georgi and Glashow (*Phys. Rev. Lett.* **32**, 438; 1975) and investigated in some detail by Ellis and collaborators at CERN, Geneva. A natural consequence of such a grand unification is that quarks (which experience QCD) and leptons (which only experience the electroweak force) are related. For example, if Nature contained a charged lepton of mass 10^{15} GeV (the 'grand unification mass' where all interactions have the same strength) then it is predicted that there would also exist a quark of charge $-\frac{1}{3}$ at that same mass. In practice the Universe is very cold, and particles have masses of order 1 GeV rather than 10^{15} GeV. Ellis and co-workers showed that as the lepton or quark mass comes down from 10^{15} GeV to 1 GeV, so the quark to lepton mass ratio increases. As an example consider the following.

The ratio of strange quark to muon mass is known to be about five (0.5 and 0.1 GeV respectively). After the discovery of the τ lepton in 1975 one necessarily predicted the existence of a 'bottom' quark in the SU(5) framework. The τ is about 2 GeV in mass, hence 'nearer' to 10^{15} GeV than the 0.1 GeV muon, with the consequence that the ratio of bottom quark to τ mass will be nearer to unity than was the ratio of strange quark to muon. Ellis *et al.* calculated this and predicted a mass of about 5 GeV for the bottom quark (about twice that of the τ lepton). The lightest meson built from a bottom quark (b) and its antiquark ($\bar{b}$) would therefore be expected at about

10 GeV. The discovery of Y at 9.46 GeV, made from bb (see *News and Views* **275**, 267; 1978) is in remarkable accord with this prediction.

Another interesting feature of grand unified theories is that the intimate relationship between quarks and leptons implies that quarks can transmute into leptons, albeit very rarely. A consequence of this is that the proton, which is made from three quarks, will not be stable; there is a probability that it will decay into three leptons. Estimates for the lifetime of the proton vary but it could be as 'short' as 10^{33} years.

As the Universe itself is only of order 10^{10} years old, this prediction may seem rather esoteric. However, Avogadro's number enables one to study vast numbers of protons and experimentally a lower limit of about 10^{30} years has been obtained for the proton's lifetime (Reines *et al. Phys. Rev. Lett.* **34**, 493; 1974). Improvement of this is feasible and so it is possible that tests of some of these ideas may be forthcoming. Several authors have recently pointed out that proton instability could be an important ingredient in quantitatively understanding the observed excess of matter over antimatter in the Universe.

Some byproducts of grand unification ideas may soon be clarified at the new high energy electron-positron collider PETRA at Hamburg. In the SU(5) and SO(10) super group schemes it is predicted that a 'top' quark must exist, charge $\frac{2}{3}$ of the proton charge, to complete the symmetry of leptons and quarks. In the scheme based on the exceptional group E_6 , however, it is predicted that you cannot have such a quark but that instead a quark with charge $-\frac{1}{3}$ will exist. Hence a search for this (sixth flavour) quark promises significant consequences for theory.

Whichever particular scheme for superunification you buy, you find the prediction of a sixth flavour of quark. Mass estimates vary somewhat but about 20 GeV seems popular. This is tantalisingly close to the maximum energy that PETRA and its Californian counterpart PEP can attain.

In addition, the quantitative successes of grand unification (such as the prediction of the b quark mass) seem to require that no more than six or eight flavours of quark and of leptons can exist. Therefore it is possible that the next generation of accelerators will reveal the end of lepton-quark spectroscopy.

If QCD can be confirmed as the correct theory of the strong interaction (see *News and Views* **277**, 349; 1979) then it seems that we will indeed have all the ingredients to hand to build a successful grand unified theory.

The menu of quark and lepton flavours and the profound similarity (and subtle differences) between quarks and leptons and also their relative masses seem already becoming more clearly understood. It seems possible that a successful grand unification might also explain the various dimensionless parameters that pervade our Universe, like α the fine structure constant, the magnitude of the Cabibbo mixing angle and the weak-electromagnetic (Weinberg) mixing angle. The possibility that the proton is not stable is also an amusing consequence.

At the centenary of Einstein's birth, this seems to be the direction in which high energy theory is moving. However, the role of gravity and its incorporation in ultimate unification, Einstein's great unsolved problem, is still unclear. Now that superunification energies approaching the Planck mass are being discussed, it seems probable that we are approaching a point where ideas in gravity and high energy physics will have to fuse if we wish to approach a full understanding of how the present cool unsymmetrical Universe froze out from the big bang. □

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High level nuclear waste isolation: borosilicate glass versus crystals

from G. de Marsily

IN a paper in this issue of *Nature* (page 219) Ringwood *et al.* advocate the use of an artificial crystalline material in which to immobilise the solid waste arising from the reprocessing of nuclear reactor fuel rods. He proposes to incorporate solid wastes in an artificial rock ('SYNROC') composed essentially of three minerals: hollandite, perovskite and zirconolite. According to Ringwood, up to 10% of waste could be incorporated into SYNROC; this material would be produced by hot-pressing the waste and ingredients at 1,200–1,300 °C, thus obtaining crystallisation, at a reasonable cost. This solution is clearly a serious potential competitor to the more usual glassification concept, which has been studied for more than 10 years, mainly in the UK and France, and is at present in operation in France at Marcoule.

As a geologist, I am concerned with

the leachability and stability of the waste in a geological formation, which will form the final barrier between the waste and our environment. From this viewpoint what are the differences between SYNROC and glass.

In their original form, the wastes produced by the reprocessing plants are liquid. Early projects suggested that this liquid be injected directly into the ground (either into a deep permeable layer, with immobile fluids, or into a cavity which would subsequently melt, due to the heat produced by the waste). Such projects have been disregarded, the radioactive elements being too readily available for transport by the ever present groundwater.

Incorporation of the calcined waste into a solid matrix then began to be considered. Many materials have been proposed, including glass, ceramic, cermets, metals, mica, feldspar, synthetic uraninite or thoriamite, and now SYNROC. So far glass has probably received most attention and given the best results. The leachability of glass depends on at least three distinct mechanisms: (1) dissolution of the borosilicate glass matrix in the water (in a salt environment, the solubility of silica increases, so that the thermally-driven fluid inclusions in the salt might have an important effect on the integrity of the glass); (2) molecular diffusion of the elements through the amorphous matrix, towards the surrounding solution, and (3) thermal diffusion (soret effect) of the elements in the glass, under the strong thermal gradients induced in the matrix by the heat produced by the waste. These three factors are also temperature dependent.

As we have discussed previously (Marsily *et al.* *Science* **197**, 519; 1977), it becomes more and more obvious that the glassified waste will have to be stored in a cooling facility for 50 years or so after the extraction of the fuel from the reactor, before final disposal in a geological formation can be considered. This interim storage can perhaps take place in the final repository, but with a cooling mechanism (ventilation for instance). This will keep the temperature in the geological formation under 100 °C at 500 m depth, with a reasonable density of waste in the glass (of the order of 10 to 20%), and of glass in the geological formation (producing a heat load of the order 50 kW ha⁻¹ or less).

After 50 yr, the waste will produce 5 kW of heat per m³ of glass, at a 20% waste load. The rate of heat production 'decays' with a 'half life' of approximately 30 yr. It is of course

possible to reduce this rate by decreasing the waste load in the glass, but then the quantity of glass to be disposed of increases, and so does the area of the repository, the costs and so on. The suggested temperature of less than 100 °C will remain more or less steady for 1,000 yr due to both dissipation of heat in the rock and heat production decay. Accepting a temperature higher than 100 °C makes the safety evaluation of the repository much more uncertain (as a result of higher leach rates and mechanical instability of the rock and so on).

With such a temperature, and if a flow of water can reach the glass (that is, assuming the geological formation is not totally impervious and the container itself is not a good barrier), conservative estimates of the time needed to remove all the radioelements from the glass are in the range of a few thousand years. Much better results can be obtained if very little water flows through the repository, because the dissolution of the silica from the glass and the diffusion by the concentration gradients are then reduced; the leachability is also reduced if the silica content is increased, and the waste load reduced (for example to 10%, as in SYNROC). Note that other artificial barriers can also be added around the waste.

Now, what about the stability of the glass? The leach rates which I have considered above already assume that the glass matrix has suffered from thermal fracture as the canisters are decontaminated and cooled after fabrication. A factor of 20 is generally accepted for the increase in area per unit volume with respect to an undisturbed glass block of 150 l. Can other mechanisms increase this area? Many experiments, including resistance to radiation, with increased quantities of α -emitters (²⁴¹Am, ²⁴⁴Cm) to simulate the passage of 1,000 yr in 2 yr have, so far, given very good results. (If ²³⁸Pu is used instead of ²⁴¹Am, however, the mechanical resistance of the glass might be drastically reduced.) Only the possibility of crystallisation is considered dangerous, and this is one of the reasons why temperature should be kept as low as possible in the repository. Even then, crystallisation of the glass does not mean disaggregation. Phase separation (creation of a soluble molybdate phase) has been reported for some types of glass, but only during processing, and has not been suggested as a potential cause of instability.

However, it is well-known that artificial and natural glasses (obsidian, for example) brought to a high temperature and pressure can be metamorphosed into a clay-type material. That is what Ringwood has done: he brought samples of glass and SYN-

ROC to 350–400 °C and 1,000 bar, and found that the glass disintegrated (whereas SYNROC resisted). I have already discussed temperature, now what about pressure? The repository will lie at a depth of 500–1,000 m. After emplacement, it is generally agreed that it will be sealed with a buffer material (such as sand and bentonite, as proposed by the Swedes) and that grouting will be made to minimise the pore space of this buffer material. At the most, this grouting will be made at the lithostatic pressure, that is, of the order of 250 bar at 1,000 m. Now it is questionable whether the waste will be subjected to this pressure, or only to the hydrostatic pressure (for example, 100 bar). In any case, the pressure and temperature used by Ringwood seem inappropriate. Twenty-four hours at high temperature and pressure will never be equivalent to several thousand years in less severe conditions: the results obtained by Ringwood are as yet insufficient to prove that glass should be eliminated as a possible confining matrix.

On the other hand, the use of a crystalline structure instead of an amorphous matrix has always been attractive to geologists: elements trapped in a crystalline network are much less mobile, provided that the crystalline mineral has a very low solubility. This became evident in the study of the Oklo natural reactor which 'burnt' uranium approximately 2 billion years ago, in Gabon. Most of the radioactive 'waste' produced during the fission reaction has been found 'trapped' inside the crystalline structure of the uraninite mineral which formed the ore body. On the other hand, the elements which could not fit into the crystalline lattice of uraninite (because of a too small or too large ionic diameter) diffused and were apparently leached away.

One may then ask the following questions. Can the three minerals constituting SYNROC host all the radioelements present in the waste, and retain them with the same strength? Apparently only Cs, Sr and U have been actually tested on SYNROC B. Can a reasonable amount of waste (10% for example) be integrated in SYNROC, the present test having been done only with 1% of Cs, Sr and U? Is the stability and low leachability of SYNROC proved under more realistic conditions (water with low pH and more complex chemical composition than just NaCl, as may be found in a granitic environment)? Is radiation damage still insignificant for a 10% load of waste? The results obtained at the Battelle and Pennsylvania State University on a similar type of material ('supercaline') give approximately the same leach-

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ability as glass. And one must remember that stability of SYNROC at high temperatures and pressures does not automatically prove stability at other thermodynamic condition (consider for instance the weathering of granite). Is the industrial production of SYNROC as easy as Ringwood suggests? I confess to being slightly worried, for instance, over the control of the development of the crystalline phase inside the canister. This seems necessary in view of one result obtained by Ringwood, where insufficient mixing of the ingredients caused the appearance of an unknown 'accessory phase' rich in caesium, which was subsequently 'removed during leaching.' Also, the actual inclusion of ruthenium in hollandite is highly questionable in the process, given the high volatility of this element. One might also ask whether SYNROC can accept variations in composition of the waste (segregation of the liquid in the tanks) and the dissolution residues.

Nevertheless, the development of a confining matrix which would be stable for several million years in any environment is most desirable. If this could be achieved, the burden of proof of the safety of a repository would be transferred from the geologist to the manufacturer of SYNROC.

As a geologist, I cannot but support research in such a direction, as long as it is not used as an argument to delay further the search for a suitable geological formation which could contain the waste securely for a very long period of time. □

Cellulose microfibrils— an end in sight?

CELLULOSE is the most abundant macromolecule in the world; the figure usually quoted for its annual production is 10^{11} tonnes. Despite the obvious economic importance that such a figure implies, research into cellulose formation has so far failed satisfactorily to explain the way in which the functional unit of cellulose—the microfibril—is made. Viewed at its simplest, the microfibril is a crystalline association of long chains of β 1–4 linked glucose units, surrounded by a region of more amorphously associated chains. The whole structure is 6–20 nm wide, depending on its source, and intact microfibrils are of indeterminate length, certainly several microns long. The higher plant cell wall consists of a network of microfibrils associated with a matrix of other polysaccharides and proteins.

The formation of the microfibril can

be regarded as a two-stage process. The first stage is the biochemical formation of the polyglucan chains. Sugar units are added to the growing polymer as nucleotide complexes: the details of this process, and in particular the sites at which it occurs within cells are as yet unclear. The second process is the physical formation of the crystalline microfibril from the polyglucan. Here there are two rather distinct hypotheses to choose from.

The first supposes that the biochemical and physical processes occur more or less simultaneously, in other words that sugar units are added on the end of a pre-existing microfibril. Evidence for this is ultrastructural—the visualisation of ordered arrays of particles at the plasma membrane surface of algae having highly ordered cellulose walls. The particles are supposed to represent enzyme complexes engaged in the synthesis of the polyglucan chains. The evidence is persuasive, particularly since it can be further supposed that ordering of wall microfibrils might be mediated by movement of such terminating enzyme complexes within the surface of the fluid membrane. Attempts to show similar particulate complexes in higher plants however have for the most part been unsuccessful, although recent work using freeze-fracture techniques has been claimed to show terminal globuli associated with microfibril ends (Willison & Grout *Planta* **140**, 53; 1978).

The alternative possibility is that the polyglucan first produced biochemically is in a soluble form, and that this intermediate associates to form the microfibril in a process not requiring direct structural linkage with the plasma membrane. This hypothesis has been recently examined by Colvin and his group, working with cellulose-producing bacteria (Colvin & Leppard *Can. J. Microbiol.* **23**, 701, 1977; Colvin *et al.* *Can. J. Biochem* **55**, 1057; 1977; Sowden & Colvin *Can. J. Microbiol.* **24**, 772; 1978). Colvin presents two lines of evidence in support of the idea that the biochemical and physical events of microfibril formation may not occur simultaneously. In structural terms, the authors describe a 'nascent' fibril, which is believed to represent an early stage in the formation of the classical microfibril. The nascent fibril has a diameter of up to 10 times that of the classical microfibril, and it consists of a dense crystalline core surrounded by an amorphous sheath which comprises most of the fibril's diameter. The nascent fibril is converted into the classical microfibril by dehydration. Thus in order to visualise the nascent fibril, only those techniques can be used which do not require the specimen to be dried before it is examined. Colvin

uses rapid freezing of 'never-dried' pellicles of bacterial cellulose, followed by deep etching of the specimen *in vacuo* and replication of the resulting etched surface. If the pellicles are first air dried, nascent fibrils are not seen, and the classical microfibril is formed.

These observations imply a change in thinking about the process of microfibril formation. The presence of nascent fibrils suggests that the acceptor for the sugar units may not be the end of a classical microfibril, but rather an unconsolidated polymer, or an association of such polymers. Direct structural involvement of membranes would not be required in this scheme.

Clearly for such an hypothesis to be viable it is necessary to demonstrate the reality of the postulated soluble intermediate polyglucan. This line of attack is the subject of the second of Colvin's three papers. The properties are described of a polysaccharide found in the medium in which the bacterial cells grow. This polysaccharide is prepared by precipitation from 60% ethanol, and further fractionated using ammonium sulphate precipitation. It appears to consist of chains of β 1–4 linked glucose units with on average a single glucose side chain on every third residue of the backbone. These branches would be expected to confer water solubility without affecting the basic linear shape of the molecule. As yet there is no direct evidence that this disperse family of polyglucans does in fact act as an intermediate in cellulose microfibril formation. Indeed, the branched structure of the polymer introduces a new complication in the form of an additional enzymic step to remove the single glucose side chains before crystallisation could take place. However, this work and its implications are at the very least sufficiently interesting to make a critical re-examination of the more established hypothesis worthwhile.

The key may after all lie with higher plant cells. Cellulose synthesis has been well studied biochemically in higher plants, but microfibril formation is naturally masked by the presence of a pre-existing cell wall. Now that the conditions for the formation of cell walls by isolated protoplasts are better understood, the time may be right to exploit this system in a more detailed way to examine *de novo* microfibril formation in higher plants. And of course, should Colvin's model prove to be applicable to cellulose formation in general, radical new thinking will be required to explain the differences in wall development which accompany cell differentiation in the higher organisms. □

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Eddington on Einstein's new theory

In 1916 Arthur Eddington, at the age of 34, was Plumian Professor of Astronomy at Cambridge, director of the university observatory and Secretary of the Royal Astronomical Society. This piece, contributed for the then equivalent of News and Views was one of the earliest simplified expositions of the General Theory in English. The author's name was inscribed over the first paragraph by the editor, Sir Norman Lockyer.

GRAVITATION AND THE PRINCIPLE OF RELATIVITY.

ACCORDING to the principle of relativity in its most extended sense, the space and time of physics are merely mental scaffolding in which for our own convenience we locate the observable phenomena of Nature. Phenomena are conditioned by other phenomena according to certain laws, but not by the space-time scaffolding, which does not exist outside our brains. As usually expressed, the laws of motion and of electrodynamics presuppose some particular measurement of space and time; but, if the principle is true, the real laws connecting phenomena must be independent of our framework of reference—the same for all systems of co-ordinates. Of course, it may be that phenomena are conditioned by something outside observation—a substantial æther which plays the part of an absolute frame of reference. But the following considerations may show that the ideal of relativity is not unreasonable. Every observation consists of a determination of coincidence in space or time. This is sufficiently obvious in laboratory experiments; and even the crudest visual observation resolves itself into the coincidence of a light-wave with an element of the human retina. If, then, we trace the path of adventure of a material particle, it intersects in succession the paths of other particles or light-waves, and these intersections or coincidences constitute the observable phenomena. We can represent the course of Nature by drawing the paths of the different particles—on a sheet of paper in a two-dimensional case. The essential part of the diagram is the order of the intersections; the paths between the intersections are outside observation altogether, and are merely interpolated. The sequence of phenomena will not be altered if the paper is made elastic and deformed in any way, because the serial order of the intersections is preserved. This deformation of the paper corresponds to a mathematical transformation of the space in which for convenience we have located the phenomena.

Until recently the application of the principle of relativity was limited to one particular transformation, namely, a uniform translation of the axes. In this case there is a wide range of experimental evidence in support of the principle. In 1915 Prof. A. Einstein¹ finally succeeded in developing the complete theory by which the postulate of relativity can be satisfied for all transformations of the co-ordinates. Gravitation plays a part of great importance in the new theory, and therein lies much of the practical interest of Einstein's work.

No attempt is made to explain the cause of gravitation—as a kink in space or anything of that nature. But the extended law of gravitation is determined, to which Newton's law is an approximation under ordinary conditions. It has long been suspected that there must be some modification of the law when the bodies concerned are in rapid relative motion; moreover, the "mass" of a moving body no longer has a unique meaning, so that a further definition, if not extension, of Newton's law is clearly needed. Now, although we do not seek a *cause* of gravitation in the properties of space, it may well happen that the *law* of gravitation is determined by these properties. The inverse-square law represents the natural weakening of an effect through spreading out in three dimensions; we may say that it is determined by the properties of Euclidean space. There is, therefore, nothing unreasonable in proceeding, as Einstein does, to examine whether a more extended law is suggested by the properties of generalised space—that is, by geometry.

The way in which gravitation enters into the discussion may be seen from the following example. Suppose an observer is in a closed lift; let the supports break and the lift fall freely. To the observer everything in the lift will now appear to be without weight; gravity has been suddenly annihilated. The acceleration of his frame of reference (the lift) is equivalent to an alteration of the gravitational field. Now an acceleration of the axes is one of the transformations contemplated by the general principle of relativity, and it is therefore necessary to allow that the gravitational field depends on the choice of co-ordinates. There is a "local" gravity, just as there is a "local" time or magnetic field depending on the co-ordinates selected.

We can now take a brief survey of Einstein's procedure. Suppose that space and time are measured by a system of co-ordinates x_1, x_2, x_3, x_4 ; x_4 is the time, but there is no need to discriminate between it and the others. If there is a gravitational field at any point, it can be abolished (as in the example of the lift) by choosing new co-ordinates x'_1, x'_2, x'_3, x'_4 accelerated with respect to the old. The necessary transformation could be specified in various ways; the way chosen involves the element of length ds , measured by coincidences with a standard scale, and therefore independent of the choice of co-ordinates. Since in the x' co-ordinates there is no gravitation to complicate matters, we can safely use the usual formula,

$$ds^2 = dx_1'^2 + dx_2'^2 + \dots$$

and this when transformed to the old co-ordinates takes the most general form,

$$ds^2 = g_{11}dx_1^2 + g_{22}dx_2^2 + \dots + 2g_{12}dx_1dx_2 + 2g_{13}dx_1dx_3 + \dots$$

The ten g 's depend on the transformation, and can

¹ Einstein, "Die Grundlage der allgemeinen Relativitätstheorie." (Leipzig: J. A. Barth, 1916.) A detailed account appears in *Monthly Notices*, 1916, No. 9, by Prof. W. de Sitter, giving the astronomical applications. See also an article by de Sitter in the *Observatory*, October, 1916.

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be used to specify it. But they do more than that; they define the original gravitational field, since they specify how it can be got rid of. They usually vary from point to point, because a different transformation is needed to eliminate gravity at different points. In the new theory the g 's are regarded as ten gravitational potentials specifying the field; and, in fact, one of them, g_{44} , is approximately the same as the Newtonian potential ϕ , except for a factor.

The inverse-square law can be expressed by the well-known differential equation $\nabla^2\phi=0$. Evidently the new law of gravitation must be a generalisation of this—an equation, or set of equations, involving the ten potentials instead of one. Also, to conform to relativity, the new equations must be unaltered by a change of co-ordinates. If new co-ordinates are used the g 's will be different, but the relations between the new g 's and new co-ordinates must be the same as those between the old g 's and old co-ordinates. The possible sets of relations which satisfy this are very limited in number. This subject, known as the theory of tensors, has been worked out very fully by Riemann, Christoffel, and others, and the possible sets of equations can be classified and enumerated. From this limited choice we have further to pick out a set of equations which will reduce to $\nabla^2g_{44}=0$ as a first approximation, and that is found to leave only one possibility. There is just one set of ten differential equations (of which, however, only six are independent) which satisfy both conditions. Einstein takes these as expressing his generalised law of gravitation. It is important to notice exactly how much of this is geometry. Geometry shows that if the equations hold for a particular set of co-ordinates, they hold for every set. We abolish the "if," and so assert a new law of Nature.

It is further necessary to consider what must be the generalised equivalent of Poisson's equation $\nabla^2\phi = -4\pi\rho$, which supplants Laplace's equation when matter is present. The extension is not difficult, since it is found that the ten equations above mentioned are the expression of a generalised principle of least action as applied to gravitational energy. Now mass is considered to be simply electromagnetic energy, and since there is no reason to believe that electromagnetic energy will behave differently from gravitational energy in regard to least action, we have only to include both forms of energy together in the equations, treating them as equivalent. It is not possible to write down here these final equations, since a very elaborate notation is needed for their expression. Though highly complicated in form, they can be applied without excessive labour to the more simple problems.

One or two of the more elementary consequences of this theory were given by Einstein some years ago. A ray of light must be bent in passing

through an intense field of gravitation; thus a star seen close to the limb of the sun during an eclipse should appear displaced $1.7''$ from its usual position. The vibrations of an atom must be slower in an intense field, so that the lines of the solar spectrum should be displaced slightly to the red as compared with terrestrial spectra. It has not yet been possible to put these predictions to a satisfactory test, and it has been left to the completed theory to furnish the first opportunity of an appeal to observation. In this the new theory has scored a most signal success, for it has cleared up the most celebrated case of discordance in gravitational astronomy.

From his generalised law of gravitation Einstein has deduced that the elliptic orbit of a planet will rotate in the direction of motion at the rate of

$$\frac{24\pi^3}{V^2} \frac{a^2}{T^2(1-e^2)} \text{ radians per revolution of the planet.}$$

Here V is the velocity of light, and a , T , e are the semiaxis, period, and eccentricity of the orbit. If v is the velocity of the planet, the amount is practically $6\pi v^2/V^2$ radians per revolution. For Mercury this works out at $43''$ per century—just the amount of the outstanding discordance between observation and theory. The theory also gives rotations for Venus and the earth, but their orbits are so nearly circular that the effect is imperceptible to observation. For Mars, with its strongly elliptic orbit, the correction is more important, and sensibly improves the accordance between theory and observation.²

It is rather difficult to grasp the fact that the same laws of Nature may hold when some bizarre system of co-ordinates is chosen. Suppose an observer A uses rectangular co-ordinates, and B, through some kink in his mind, uses polar co-ordinates without realising that he is doing anything unusual. For A a ray of light can travel along the straight line $x=\text{constant}$; but evidently it cannot travel along the circle $r=\text{constant}$, which is B's idea of a straight line. The answer is that B through his peculiar system of measurement will suppose that he is in an intense gravitational field; he will calculate the curvature in the ray of light produced by this field; and, making allowance for it, he will find that the light actually travels along its theoretical curve (*i.e.* curve for B, but straight line for A). Thus the same general laws of Nature are satisfied for B as well as for A; and it might be difficult to decide which of them had got hold of the absolute rectangular co-ordinates.

A. S. EDDINGTON.

² The discordance of the perihelion of Mercury (now removed) was nearly 30 times its probable error. Of the sixteen secular variations of the four inner planets, all are now accordant, except the node of Venus, which deviates by $4\frac{1}{2}$ times its probable error. Among sixteen residuals we should expect to find one of three times the probable error, so that the evidence for the remaining discordance is not very strong. There are besides unexplained variations of the longitudes of the moon and planets, but these are in a different category.

Albert Einstein: 14 March, 1879—18 April, 1955

A guide for the perplexed

Kenneth Brecher

This brief collection of direct and indirect quotations by or about Albert Einstein is offered as a tribute in honour of the centenary of his birth. The selection makes no attempt to be complete but does try to be representative of his attitude towards science and mathematics. As this selection is addressed to a primarily scientific audience, his views concerning his two most important creations, quantum theory and relativity, are particularly stressed toward the end.

The most beautiful experience we can have is the mysterious. It is the fundamental emotion which stands at the cradle of true art and true science. Whoever does not know it and can no longer wonder, no longer marvel, is as good as dead, and his eyes are dimmed.

from *The World as I See It*,
by A. Einstein (Philosophical
Library: New York, 1934)

I believe with Schopenhauer that one of the strongest motives that lead persons to art and science is flight from the everyday life, with its painful harshness and wretched dreariness, and from the fetters of one's own shifting desires. One who is more finely tempered is driven to escape from personal existence and to the world of objective observing and understanding. This motive can be compared with the longing that irresistibly pulls the town dweller away from his noisy, cramped quarters and toward the silent, high mountains, where the eye ranges freely through the still, pure air and traces the calm contours that seem to be made for eternity.

With this negative motive there goes a positive one. Man seeks to form for himself in whatever manner is suitable for him, a simplified and lucid image of the world, and so to overcome the world of experience by striving to replace it to some extent by this image. This is what the painter does, and the poet, the speculative philosopher, the natural scientist, each in his own way. Into this image and its formation, he places the centre of gravity of his emotional life, in order to attain the peace and serenity that he cannot find within the narrow confines of swirling, personal experience.

from *The Scientific Imagination
Case Studies* (page 231), by
G. Holton (Cambridge
University Press: Cambridge, 1978)

The most incomprehensible thing about the universe is that it is comprehensible.

in *Albert Einstein: Creator and
Rebel* (page 18), by Banesh
Hoffman (Viking: New
York, 1972; Hart-Davis,
MacGibbon: London, 1973;
Paladin: London, 1977)

Out yonder there was this huge world, which exists independently of us human beings and which stands before us like a great, eternal riddle, at least partially accessible to our inspection.

from "Autobiographical
Notes", in *Albert Einstein:
Philosopher-Scientist*, edited by
E. A. Schilpp (Harper and
Row: New York, 1959)

The essence of a man like me lies just in what he thinks and how he thinks—not in what he does or suffers.

from "Autobiographical
Notes", in *Albert Einstein:
Philosopher-Scientist*, edited by
E. A. Schilpp (Harper and
Row: New York, 1959)

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To the sphere of religion belongs the faith that the regulations valid for the world of existence are rational, that it is comprehensible to reason. I cannot conceive of a genuine scientist without that profound faith. The situation may be expressed by an image: science without religion is lame, religion without science is blind.

in *Einstein: His Life and
Times*, by P. Frank
(Knopf: New York, 1947)

Of all the communities available to us, there is not one I would want to devote myself to, except for the society of true searchers, which has very few living members at any time.

in letter to Max Born, April
29, 1924, from *The Born-
Einstein Letters*, by M. Born
(Walker: New York, 1971)

Kenneth Brecher is Professor of Physics at the Massachusetts Institute of Technology, Cambridge, Massachusetts. All quotations are reproduced with the permission of the publishers or of the Estate of Albert Einstein.

In every true searcher of Nature there is a kind of religious reverence; for he finds it impossible to imagine that he is the first to have thought out the exceedingly delicate threads that connect his perceptions. The aspect of knowledge which has not yet been laid bare gives the investigator a feeling akin to that experienced by a child who seeks to grasp the masterly way in which elders manipulate things.

in *Conversations with Einstein*,
by A. Moszkowski (Horizon
New York, 1970)

What I'm really interested in is whether God could have made the world in a different way; that is, whether the necessity of logical simplicity leaves any freedom at all.

to Ernst Straus, quoted in
*The Scientific Imagination Case
Studies*, by G. Holton
(Cambridge University Press:
Cambridge, 1978)

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I want to know how God created this world. I am not interested in this or that phenomenon, in the spectrum of this or that element. I want to know His thoughts, the rest are details.

in *Einstein: The Life and
Times*, by R. W. Clark (World
Publishing: New York, 1971;
Hodder and Stoughton:
London, 1979)

God is cunning but He is not malicious. (*Raffiniert ist der Herr Gott aber boshaft ist Er nicht.*)

inscribed at Princeton

Physics is essentially an intuitive and concrete science. Mathematics is only a means for expressing the laws that govern phenomena.

from *Lettres à Maurice
Solovine*, by A. Einstein
(Gauthier-Villars: Paris, 1956)

God does not care about our mathematical difficulties. He integrates empirically.

in *Quest* (page 279), by L.
Infeld (Gollancz: London, 1942)

How can it be that mathematics, being after all a product of human thought which is independent of experience, is so admirably appropriate to the objects of reality? Is human reason, then, without experience, merely by taking thought, able to fathom the properties of real things? In my opinion the answer to this question is, briefly, this: as far as the propositions of mathematics refer to reality,

they are not certain; and as far as they are certain, they do not refer to reality.

from "Geometry and
Experience", in *Ideas and
Opinions* (page 233), by
A. Einstein (Crown:
New York, 1954)

In the matter of physics, the first lessons should contain nothing but what is experimental and interesting to see. A pretty experiment is in itself often more valuable than twenty formulae extracted from our minds; it is particularly important that a young mind that has yet to find its way about in the world of phenomena should be spared from formulae altogether. In his physics they play exactly the same weird and fearful part as the figures of dates in Universal History.

from *Conversations with
Einstein*, by A. Moszkowski
(Horizon: New York, 1970)

What applies to jokes, I suppose, also applies to pictures and to plays. I think they should not smell of a logical scheme, but of a delicious fragment of life, scintillating with various colours according to the position of the beholder. If one wants to get away from this vagueness one must take up mathematics. And even then one reaches one's aim only by becoming completely insubstantial under the dissecting knife of clarity. Living matter and clarity are opposites—they run away from one another. We are now experiencing this rather tragically in physics.

in letter to Max Born, January
15, 1927, from *The Born-
Einstein Letters*, by M. Born
(Walker: New York, 1971)

It is in fact, nothing short of a miracle that the modern methods of instruction have not yet entirely strangled the holy curiosity of inquiry; for this delicate plant, aside from stimulation, stands mainly in need of freedom; without this it goes to wrack and ruin without fail.

from "Autobiographical
Notes", in *Albert Einstein:
Philosopher-Scientist*, edited by
E. A. Schilpp (Harper and
Row: New York, 1959)

The only rational way of education is to be an example—if one can't help it, a warning example.

To punish me for my contempt for authority, Fate made me an authority myself.

in *Albert Einstein: Creator and
Rebel* (page 24), by Banesh
Hoffman (Viking:
New York, 1972; Hart-Davis,
MacGibbon: London, 1973;
Paladin: London, 1977)

Great spirits have always encountered violent opposition from mediocre minds.

I remember a beautiful remark of his when he criticized a well-known American physicist. Einstein said he "couldn't really understand how anybody could know so much and understand so little"! Einstein always emphasized that you could know too many facts and get lost among them.

E. H. Hutten, in *Einstein:
The Man and His Achieve-
ment*, edited by G. J. Whitrow
(Dover: New York, 1973)

About ten years ago I spoke with Einstein about the astonishing fact that so many ministers of various denominations are strongly interested in the theory of

relativity. Einstein said that according to his estimation there are more clergymen interested in relativity than physicists. A little puzzled I asked him how he would explain this strange fact. He answered, a little smiling, "Because clergymen are interested in the general laws of nature and physicists, very often, are not." Another day we spoke about a certain physicist who had very little success in his research work. Mostly he attacked problems which offered tremendous difficulties.

By most of his colleagues he was not rated very highly. Einstein, however, said about him, 'I admire this type of man. I have little patience with scientists who take a board of wood, look for its thinnest part and drill a great number of holes where drilling is easy'.

Philipp Frank, "Einstein's Philosophy of Science", in *Reviews of Modern Physics*, **21**, 349 (1949)

recall a visit I once paid him on which we decided to visit the astrophysical observatory at Potsdam together. We agreed to meet on a certain bridge in Potsdam, but since I was a good deal of a stranger in Berlin, I said I could not promise to be there at the appointed time.

"Oh," said Einstein, "that makes no difference; then I will wait on the bridge."

I suggested that that might waste too much of his time.

"Oh no," was the rejoinder, "the kind of work I do can be done anywhere. Why should I be less capable of reflecting about my problems on the Potsdam bridge than at home?"

in *Einstein: His Life and Times* (page 118), by P. Frank (Knopf: New York, 1947)

I might begin with a true story that some of you may know concerning a conversation between Einstein and the French poet Valery. When Einstein first came to Paris, a well known hostess achieved the triumph of having both Einstein and Valery in her drawing-room, and arranged for a conversation between the two that everyone else could listen to. As you know, Valery's mania was to say that he was more interested in the process of creation than in the actual opus which came out of this creative process . . . and he started asking questions of Einstein about how he worked.

"How do you work, and could you tell us something of this?" Einstein was very vague about it.

He said, "Well, I don't know . . . I go out in the morning and take a walk."

"Oh", said Valery, "Interesting, and of course you have a notebook and whenever you have an idea you write it out in your notebook."

"Oh", said Einstein, "No I don't".

"Indeed, you don't?"

"Well you know, an idea is so rare."

That is really as much as I think can be said as to really great creativity.

J. Monod, in *The Creative Process in Science and Medicine*, edited by H. A. Krebs and J. H. Shelley (Elsevier: New York, 1975)

He was asked whether he thought that everything could ultimately be expressed in scientific terms. Einstein replied: "Yes, that is conceivable, but it would make no sense. It would be as if one were to reproduce Beethoven's Ninth Symphony in the form of an air pressure curve."

G. Born, in *The Creative Process in Science and Medicine*, edited by H. A. Krebs and J. H. Shelley (Elsevier: New York, 1975)

During his Zurich stay the woman doctor, Paulette Brubacher, asked the whereabouts of his Laboratory. With a smile he took a fountain pen out of his breast pocket and said: "Here".

from *Albert Einstein: A Documentary Biography*, by C. Seelig (Staples: London, 1956)

The most important tool of the theoretical physicist is his wastebasket.

told by P. Morrison

Just as it is the pride of many people never to have any time, so it has been Einstein's always to have time. I

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With his 'laboratory', 1948

Camera Press

The friendship of my thesis professor, Rudolf Ladenburg, and Einstein was a long one. They had been together in Berlin before coming to Princeton. Ladenburg liked to tell of their first meeting, about 1908, when he called upon Einstein in the Swiss Patent Office. Einstein told him that he was the first physicist that he had seen in five years. During these years Einstein had done much of his important work. He pulled out one drawer of his desk and said that it was his theoretical physics office. His duty of reading patents took little time, and he worked upon physics whenever he was free.

Y. Beers, *Am. J. Phys.*, **46**, 506 (1978)

I never think of the future, it comes soon enough.

Who would have thought around 1900 that in fifty years time we would know so much more and understand so much less . . . Nowadays, one can be happy if one is not trampled down by the stampede of the buffalos.

in *Albert Einstein and the Cosmic World Order*, by C. Lanczos (Wiley: New York, 1965)

A theory is the more impressive the greater the simplicity of its premise is, the more different kinds of things it relates, and the more extended is its area of applicability. It [thermodynamics] is the only physical theory of universal content concerning which I am convinced that,

within the framework of applicability of its basic concepts, it will never be overthrown.

from "Autobiographical Notes", in *Albert Einstein: Philosopher-Scientist*, edited by E. A. Schilpp (Harper and Row: New York, 1959)

It was therefore quite a shock when he said, "But why should anybody be interested in getting exact solutions of such an ephemeral set of equations?". I remember very well this word "ephemeral". It meant that he did not consider his gravitational equations as the last word.

by C. Lanczos, in *Einstein: The Man and His Achievement* (page 49), edited by G. J. Whitrow (Dover: New York, 1973)

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Press conference, New York, 1930

But it [general relatively] is similar to a building, one wing of which is made of fine marble (left part of the equation), but the other wing of which is built of low grade wood (right side of the equation). The phenomenological representation of matter is, in fact, only a crude substitute for a representation which would do justice to all known properties of matter.

from "Physics and Reality" (1936), reprinted in *Ideas and Opinions* (page 311), by Albert Einstein (Crown: New York, 1954)

I can, if the worst comes to the worst, still realise that the Good Lord may have created a world in which there are no natural laws. In short, chaos. But that, there should be statistical laws with definite solutions, i.e., laws which compel the Good Lord to throw the dice in each individual case, I find highly disagreeable.

in *Albert Einstein: A Documentary Biography*, by C. Seelig (Staples: London, 1956)

You believe in a dice-playing God and I in perfect laws in the world of things existing as real objects, which I try to grasp in a wildly speculative way.

from letter to Max Born, quoted in *Albert Einstein: Philosopher-Scientist* (page 176), edited by E. A. Schilpp (Harper and Row: New York, 1959)

The present theory of relativity is based on a division of physical reality into a metric field (gravitation) on the one hand, and into an electromagnetic field and matter on the other hand. In reality space will probably be of a uniform character and the present theory be valid only as a limiting case. For large densities of field and of matter, the field equations and even the field variables which enter

into them will have no real significance. One may not therefore assume the validity of the equations for very high density of field and of matter, and one may not conclude that the "beginning of the expansion" must mean a singularity in the mathematical sense. All we have to realise is that the equations may not be continued over such regions.

in *The Meaning of Relativity* (page 129), fifth edition, by Albert Einstein (Princeton University Press: New Jersey, 1956)

Quantum mechanics is certainly imposing. But an inner voice tells me that it is not yet the real thing. The theory says a lot, but does not really bring us any closer to the secret of the 'old one'. I, at any rate, am convinced that He is not playing at dice.

from a letter to Max Born, December 4, 1926, from *The Born-Einstein Letters*, by M. Born (Walker: New York, 1971)

The Heisenberg-Bohr tranquilizing philosophy—or religion?—is so delicately contrived that, for the time being, it provides a gentle pillow for the true believer from which he cannot very easily be aroused. So let him lie there.

in a letter to E. Schrödinger, May 31, 1928, from *Letters on Wave Mechanics* (page 31), edited by K. Przibram (Philosophical Library: New York, 1967)

Is it conceivable that a field theory permits one to understand the atomistic and quantum structure of reality? Almost everybody will answer this question with "no". But I believe that at the present time nobody knows anything reliable about it. This is so because we cannot judge in what manner and how strongly the exclusion of singularities reduces the manifold of solutions...

One can give good reasons why reality cannot at all be represented by a continuous field. From the quantum phenomena it appears to follow with certainty that a finite system of finite energy can be completely described by a finite set of numbers (quantum numbers). This does not seem to be in accordance with a continuum theory, and must lead to an attempt to find a purely algebraic theory for description of reality. But nobody knows how to obtain the basis of such a theory.

in *The Meaning of Relativity* (page 165), fifth edition, by Albert Einstein (Princeton University Press: New Jersey, 1956)

You imagine that I look back on my life's work with calm satisfaction. But from nearby it looks quite different. There is not a single concept of which I am convinced that it will stand firm, and I feel uncertain whether I am in general on the right track . . . I don't want to be right . . . I only want to know whether I am right.

from *Lettres à Maurice Solovine*, by Albert Einstein (Gauthier-Villars: Paris, 1956)

One thing I have learned in a long life: that all our science, measured against reality, is primitive and childlike—and yet it is the most precious thing we have.

frontispiece to *Albert Einstein: Creator and Rebel*, by Banesh Hoffman (Viking: New York, 1972; Hart-Davis, MacGibbon: London, 1973; Paladin: London, 1977)

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articles

Immobilisation of high level nuclear reactor wastes in SYNROC

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The elements occurring in high-level nuclear reactor wastes can be safely immobilised by incorporating them within the crystal lattices of the constituent minerals of a synthetic rock (SYNROC). The preferred form of SYNROC can accept up to 20% of high level waste calcine to form dilute solid solutions. The constituent minerals, or close structural analogues, have survived in a wide range of geochemical environments for periods of 20–2,000 Myr whilst immobilising the same elements present in nuclear wastes. SYNROC is unaffected by leaching for 24 h in pure water or 10 wt % NaCl solution at high temperatures and pressure whereas borosilicate glasses completely decompose in a few hours in much less severe hydrothermal conditions. The combination of these leaching results with the geological evidence of long-term stability indicates that SYNROC would be vastly superior to glass in its capacity to safely immobilise nuclear wastes, when buried in a suitable geological repository. A dense, compact, mechanically strong form of SYNROC suitable for geological disposal can be produced by a process as economical as that which incorporates radwaste in borosilicate glasses.

SEVERAL countries propose to solidify the high level nuclear wastes arising from reprocessing of nuclear reactor fuel rods, and to bury the solids in suitable geological repositories. Because of the intensely radioactive nature of the waste (Table 1) this material must be prevented from reaching the biosphere for a period which may be as long as 1 Myr. This requires, among other things, that the waste be solidified in a form possessing extreme stability and resistance to leaching and alteration in appropriate geological environments.

The most popular procedure advocated by the nuclear power establishment during the past 25 yr has been to incorporate the radwaste into a borosilicate glass. The product typically contains ~25% of radwaste. There are, however, some serious disadvantages of this procedure which have become recognised only comparatively recently. Borosilicate glasses readily devitrify when subjected to the action of water and steam at elevated pressures and temperatures, for example at 300–400 °C and 300–1,000 bar^{2,3}. Pressure–temperature conditions in the lower end of this range may well be encountered by glass cylinders after burial in geological repositories. Moreover, it would be extremely difficult to design a repository in which groundwater can be prevented from gaining access to radwaste. (This includes

repositories in salt formations⁴). There is thus a high probability that glass cylinders will devitrify within relatively short intervals after burial. Devitrification in these conditions can cause a drastic increase in effective surface area and in leachability of certain highly dangerous species such as caesium and the actinides³.

It is unlikely that the problem can be avoided by reducing the proportion of waste and ageing the glass for 30 yr to achieve lower temperatures⁵. Detailed studies³ show that the mechanism of alteration is complex. One of the mechanisms involves diffusion of water into the glass structure. This precedes the actual devitrification and causes the interior of the glass to lose its strength. Another mechanism involves selective leaching of components from regions where devitrification is occurring, thereby producing a disintegrated material with a high degree of heterogeneity. Moreover, the rate of alteration is non-linear with time. Because of the complexity of the reaction mechanisms, it is not possible to predict the stabilities of glasses with respect to devitrification in geological environments at low temperatures in the vicinity of 100–150 °C.

Table 1 Principal components of a typical calcine (radwaste)¹

		Mol %
Rare earths (REE elements)		26.4
Zr		13.2
Mo		12.2
Ru		7.6
Cs	Fission products	7.0
Pd		4.1
Sr		3.5
Ba		3.5
Rb		1.3
U + Th	Actinides	1.4
Am + Cm + Pu + Np		0.2
Fe	Processing contaminants	6.4
(PO ₄)		3.2
Na		1.0
Others (mainly Tc, Rh, Te, I and processing contaminants (including Ni, Cr)		9.0

Incorporation of radwaste in crystalline materials

McCarthy^{1,6} and Roy⁷ have previously recognised some of the uncertain aspects of immobilisation in glasses and have proposed that radwaste be incorporated in ceramic materials composed of crystalline phases. A phase assemblage of this

nature would be much closer to thermodynamic equilibrium than glass, so that the risks arising from devitrification of glasses would be avoided. These authors also described the basic crystal chemical principles underlying the incorporation of radwaste elements in crystalline phases.

There is, of course, an enormous range of crystalline phases, phase assemblages and methodologies which can be conceived for the purposes of storing radwaste elements. The particular strategy chosen by McCarthy led to the formulation of a series of materials designated by the general name of 'Supercalcine'. These materials are produced by adding ~50% of the oxides of Si, Ca, Al and Sr to the high level waste before calcination (Table 2). The above components are added in carefully-defined proportions so that during calcination, they will react with the components of radwaste to form a specific assemblage of desired crystalline phases. The preferred assemblage for waste derived from light-water reactors contains phases possessing apatite, fluorite, scheelite, pollucite and spinel-type structures.

McCarthy and Roy deserve credit for recognising at an early stage the disadvantages of glass as an immobilisation medium, and for perceiving the advantages of incorporating radwaste within crystalline phases. However, it is now clear that their Supercalcine strategy does not achieve many of the potential advantages of immobilising radwaste within crystalline phases. A detailed discussion of weaknesses of the Supercalcine strategy in this respect is given in refs 3 and 4.

An alternative method of radwaste immobilisation in crystalline materials was described by Ringwood⁴. This was based on incorporation of radwaste elements as dilute solid solutions in the constituent minerals of a synthetic rock—SYNROC. The preferred method of producing this SYNROC was to melt a mixture of TiO₂, BaO, SiO₂, ZrO₂, Al₂O₃, CaO and K₂O (Table 2, column I) at 1,300 °C and cool slowly to produce a synthetic rock composed of the minerals 'hollandite' BaAl₂Ti₆O₁₆, perovskite CaTiO₃, zirconolite CaZrTi₂O₇, Ba-felspar BaAl₂Si₂O₈, kalsilite KAlSiO₄ and leucite KAlSi₂O₆. Alternatively, this mineral assemblage could be produced by hot-pressing the above composition in subsolidus conditions. About 10% of radwaste calcine would be intimately mixed with the above SYNROC components before melting and crystallisation. Ringwood showed that nearly all the elements present in high-level waste (Table 1) were incorporated as dilute solid solutions within the individual minerals of SYNROC, and could therefore be immobilised for long periods when SYNROC was buried in a suitable geological environment.

The actual composition and mineral assemblage of SYNROC (Table 2) differs drastically from that of Supercalcine. There is also an important difference in concept. Because of the large proportion of radwaste (>50%) in Supercalcine, the radwaste

elements largely control the nature of the crystalline phase assemblage. This greatly restricts flexibility and yields a phase assemblage which possesses some undesirable characteristics^{3,4}. However, SYNROC contains only ~10% of radwaste. Thus, the 90% of added components controls the nature of the phase assemblage and the radwaste atomic species simply substitute in low concentrations within the crystal lattices determined by the major added components, as in nature. This provides a great deal of flexibility in selecting a crystalline phase assemblage with the most desirable immobilisation characteristics.

An important characteristic of SYNROC, therefore, is that its particular mineral assemblage (Table 2) remains essentially the same, whether the radwaste component amounts to 0%, 5%, 10% or even 20%. The advantages of this procedure are discussed more fully in refs 3 and 4.

We have since modified and simplified the original composition (termed SYNROC A in Table 2) by eliminating the silicate minerals Ba-felspar + kalsilite ± leucite. The modified version of SYNROC B is composed essentially of the minerals 'hollandite' + perovskite + zirconolite. Because of the absence of silicates, the titanium content is much higher and there is a superficial resemblance to the high-titanium ceramics developed by the Sandia Corporation⁷. However, Table 2 shows that the detailed chemical and mineralogical composition of SYNROC in fact differs fundamentally from that of the Sandia material.

Our main reason for developing SYNROC B was to incorporate radwaste caesium in the hollandite phase, which has proved to be exceptionally resistant to leaching. This was found to require the removal of kalsilite, leucite and Ba-felspar from the assemblage. Because of the absence of silicates, the melting temperature of SYNROC B is much higher than that of SYNROC A. Accordingly, we prefer to produce SYNROC B by hot-pressing and recrystallisation in the solid state at ~1,300 °C, as discussed subsequently.

Mineralogy of SYNROC B

When the SYNROC B bulk composition (Table 2, column 2) is hot-pressed at 1,200–1,400 °C, the minerals are found to possess hollandite, perovskite and zirconolite structures. Typical compositions of these minerals are given in Table 3.

'Hollandite': natural hollandite has the formula BaMn₈O₁₆, where manganese can occur in 2+, 3+, and 4+ valence states. There are, however, a large number of structural isotypes, including BaAl₂Ti₆O₁₆, in which the Mn²⁺ and Mn⁴⁺ ions of natural hollandite are replaced by Al³⁺ and Ti⁴⁺. The most abundant mineral in SYNROC A and B is BaAl₂Ti₆O₁₆. In subsequent discussions, this phase will be referred to as 'hollandite'.

Table 2 Comparison of SYNROC with alternative ceramic waste forms

	I SYNROC A	II SYNROC B	III Typical Supercalcine*	IV Sandia ceramic†
Radwaste (wt %)	10	10	50	~25
Inert additives (wt %)	90	90	50	~75
	Composition of inert additives (wt %)			
SiO ₂	13	—	68	Minor
TiO ₂	33	57	—	~90
ZrO ₂	10	11	—	—
Al ₂ O ₃	16	10	11	Minor
CaO	6	13	19	—
BaO	17	9	—	—
SrO	—	—	2	—
Na ₂ O	—	—	—	Minor
K ₂ O	5	—	—	—
Mineral structures	Hollandite, perovskite, zirconolite, Ba-felspar, kalsilite, leucite	Hollandite, perovskite, zirconolite	Scheelite, cubic zirconia, spinel, apatite, corundum, pollucite	Rutile, metal, cubic zirconia, pollucite, Gd ₂ Ti ₂ O ₇ , amorphous silica

* From ref. 1. † From ref. 7.

Table 3 Compositions of coexisting phases in SYNROC B, doped with ~1% each of Cs₂O, UO₂ and SrO

	'Hollandite'	Perovskite	Zirconolite
TiO ₂	72.0	56.5	48.4
ZrO ₂	0.3	0.8	30.6
UO ₂	<0.1	0.8	3.8
Al ₂ O ₃	13.1	0.4	2.6
NiO	0.9	<0.1	<0.1
BaO	12.0	<0.2	<0.2
SrO	<0.1	2.5*	1.3
CaO	<0.2	39.2	13.8
Cs ₂ O	1.5	<0.1	<0.1
Total	99.7	100.2	100.5

* In other experiments, up to 7.5% SrO has been found in perovskite, with smaller amounts in zirconolite.

The hollandite structure is closely related to that of rutile, but differs in having tunnels parallel to the vertical axis in which very large atoms such as K, Rb, Cs, Ba, Sr and Pb may be stably bound. Moreover, many elements including Mg, Co, Ni, Cu, Cr, Fe, Mn, Mo, Rh, Ru, Sn and Zr are capable of replacing Al and Ti in the octahedral lattice sites. Because of the great flexibility and range of permitted ionic substitutions, and its resistance to leaching, 'hollandite' is a key component of SYNROC. We have also synthesised pure hollandite forms of Cs₂Al₂Ti₆O₁₆, K₂Al₂Ti₆O₁₆, and SrAl₂Ti₆O₁₆ and demonstrated the existence of extensive solid solutions with BaAl₂Ti₆O₁₆. Perovskite, CaTiO₃: this mineral is capable of taking an extremely wide range of other elements into stable solid solution. The elements which are capable of replacing calcium in its lattice site include Sr²⁺, Ba²⁺, Na⁺, all rare earths (REE)³⁺, Y³⁺, Cd³⁺, Cm³⁺, Am³⁺, Pu³⁺ whilst the elements capable of entering the titanium lattice site include Nb⁵⁺, Zr⁴⁺, Mo⁴⁺, Pu⁴⁺, Rh⁴⁺, U⁴⁺, Sn⁴⁺, Ru⁴⁺, Fe³⁺, Cr³⁺, Al³⁺. Many more substitutions are known, but only those relevant to radwaste immobilisation have been mentioned. Zirconolite, CaZrTi₂O₇: this mineral possesses a distorted pyrochlore structure. A large number of pyrochlore isotypes are known and a wide range of atomic substitutions are possible. Thus, the eight-fold sites can be occupied by Na, K, Ca, Sr, Ba, Pb, Y, REE, Bi, Zr, U, Th, whilst Ta, Nb, Ti, Fe and Mn can substitute in the six-fold sites.

Incorporation of radwaste elements in SYNROC minerals

The relative proportions of different elements present in high-level wastes are given in Table 1. We have doped samples of SYNROC A and SYNROC B with 1–3% of non-radioactive isotopes of most of these elements. These compositions were then crystallised by appropriate heat-treatments at 1,150–1,350 °C and distributions of the tracers between coexisting phases were measured by electronprobe microanalyses. These experiments showed that nearly all the radwaste elements in Table 1 readily enter and form homogeneous solid solutions with the SYNROC minerals, as discussed elsewhere^{3,4}. The results are summarised in Table 4, showing which phase is the host for a particular element. On general crystal chemical grounds, we can be confident that the remaining rare earth elements will behave similarly to La, Gd and Y whilst the actinide elements (including plutonium, americium and curium) will behave similarly to uranium and/or the rare earths, entering zirconolite, and, to a lesser extent, perovskite. In SYNROC B, rubidium will doubtless behave similarly to potassium and caesium, thereby entering the hollandite phase. Phosphorus is found to combine with Ba and Ca to form minor amounts of an orthophosphate phase (Ba, Ca)₃(PO₄)₂, whilst palladium and tellurium are reduced to the metallic state (Te as NiTe).

It is remarkable that almost the entire range of radwaste elements can be incorporated in only three crystalline phases, which are also thermodynamically compatible. This is a consequence of the structures and crystal chemical properties of these phases which permit an extremely wide range of ionic

substitutions. In principle, it would be possible to omit the perovskite phase, as the radwaste elements occurring in perovskite can also enter 'hollandite' and zirconolite. However, we retain perovskite in the SYNROC B assemblage because it preferentially accommodates ⁹⁰Sr, one of the most dangerous of radwaste isotopes. With this particular mineralogy, each of the three SYNROC B minerals thus accommodates preferentially a particularly hazardous radwaste element or class of elements—caesium enters 'hollandite', strontium enters perovskite, whilst actinide elements mainly enter zirconolite.

Accelerated leaching tests on SYNROC and glasses

We have investigated the relative stabilities of different waste forms by subjecting them to the action of water and aqueous solutions at elevated pressures and temperatures. The samples used were in the form of short cylinders with diameters of 2–4 mm. These were sealed in silver tubes, together with 12 times their volume of water or 10 wt % NaCl solution. After sealing, the tubes were placed in hydrothermal apparatus and subjected to the desired pressure–temperature conditions.

Borosilicate glasses possessing compositions proposed for radwaste immobilisation were completely devitrified and often disintegrated when subjected to the effects of pure water and 10 wt % NaCl solutions at temperatures of 350–400 °C and at 1,000 bar for 24 h. Extensive losses of caesium (50–95%), uranium (30–90%) and other cations were also observed.

In contrast, samples of SYNROC A (doped with 1% of Cs) remained unaltered when subjected to the effects of pure water at 1,000 bar, 24 h, and at temperatures up to 600 °C, demonstrating superiority to glass in this respect. However, when subjected to the action of 10% NaCl solution in the same conditions, kalsilite and leucite phases showed partial and extensive decomposition at 400 and 500 °C respectively, accompanied by loss of caesium. At 600 °C, these phases were completely decomposed and replaced by sodalite, with total loss of caesium. A similar series of experiments conducted on a sample of SYNROC A containing accessory pollucite (CsAlSi₂O₆) which is the host for caesium in Supercalcine and in the Sandia ceramic (Table 2, Columns III and IV) showed that this mineral was similarly unstable when subjected to the action of NaCl solutions in the above conditions. It seems then, that framework-type silicates such as pollucite, leucite and kalsilite may not be ideal for immobilising radwaste caesium in the presence of groundwaters containing dissolved sodium.

Samples of SYNROC B containing 1% each of Cs, Sr and U were next treated with pure water and 10% NaCl solutions in similar conditions to those used on glass and SYNROC A, but at 400, 500, 600, 700 and 800 °C. There was no significant alteration. The individual mineral phases remained stable and there was no measurable loss of Cs from 'hollandite', U from zirconolite or of Sr from perovskite. Moreover, there was no indication of entry of Na from the NaCl solution into the 'hollandite'. Similar leaching experiments were also carried out at 900 and 1,000 °C and at 5,000 bar for 24 h. In the 900 °C

Table 4 Summary of experimental data showing host phases for principal high-level waste elements introduced into SYNROC B

'Hollandite' BaAl ₂ Ti ₆ O ₁₆		Zirconolite CaZrTi ₂ O ₇	Perovskite CaTiO ₃
Cs ⁺	Mo ⁴⁺	U ⁴⁺	Sr ²⁺
K ⁺	Ru ⁴⁺	Zr ⁴⁺	(U ⁴⁺)
(Na ⁺)	Rh ³⁺	Y ³⁺	(Y ³⁺)
Ba ²⁺	Fe ³⁺	Gd ³⁺	(Gd ³⁺)
	Cr ³⁺	La ³⁺	(La ³⁺)
	Ni ²⁺	Na ⁺	
	Fe ²⁺		

Actinide elements and rare earths preferentially enter zirconolite when this phase is present. However, they can also be accommodated by perovskite.

experiments, the 'hollandite' in the peripheral regions of the sample altered to rutile and caesium was lost into solution. However, the interior was unchanged. Zirconolite and perovskite throughout the entire sample were unaltered and retained their uranium and strontium quantitatively. At 1,000 °C, the samples were altered more extensively, but again, the central region was not affected.

The major minerals of SYNROC B exhibit a truly remarkable degree of resistance to hydrothermal alteration as compared to borosilicate glass, Supercalcine and SYNROC A. The extreme stability of these minerals justifies the conclusion that SYNROC B would constitute an excellent medium for radwaste immobilisation.

The remarkable ability of 'hollandite' to retain caesium and to resist exchange with sodium is probably a consequence of its crystal structure. Exchange can occur only through uniaxial diffusion of alkali elements through the narrow channels in the lattice parallel to the C axis. These are effectively blocked by Ba^{2+} ions which are tightly bonded because of their double charges and the caesium ions are thus locked-in. In contrast, open framework structures such as kalsilite, leucite and pollucite offer much easier three-dimensional access, thereby permitting exchange with sodium ions in solution and ultimate dissolution.

One problem encountered with the first batch of runs on SYNROC B arose from the loss of caesium from an unknown accessory phase (other than 'hollandite') during leaching. Broad-beam microprobe analyses of starting materials indicated the presence of 30–40% more caesium than could be accounted for by the 'hollandite'. This caesium was presumably present in another accessory phase which was removed during leaching. The formation of this phase was interpreted to result from incomplete equilibration during hot-pressing, due to imperfect mixing of components in the initial starting materials. This interpretation was supported by a subsequent series of four runs on modified SYNROC B compositions containing excess Al_2O_3 (13%) and, separately, excess TiO_2 (67%). The samples were mixed more thoroughly and hot-pressed at 1,350 °C, resulting in much coarser crystals and presumably, a closer approach to equilibrium. Samples of these runs were leached in 10% NaCl solutions for 24 h. No detectable losses of caesium from the bulk specimens were observed.

Radiation damage

Concern has been expressed that radiation damage to crystals of ceramic immobilisation media might enhance the solubility of radwaste elements in groundwater. Radiation damage, principally from actinide elements which emit α particles, causes disorder of the host lattice leading to the metamict state, sometimes accompanied by extensive volume expansion. However, experimental studies reviewed in ref. 4 show that, providing a radioactive element is substituting in a stable lattice site (such as U^{4+} substituting for Zr^{4+} in zircon), its solubility in aqueous solutions is not markedly increased by radiation damage. Aqueous solutions at elevated pressures and temperatures simply cause recrystallisation to the original state without loss of crystal-chemically compatible radioactive elements.

Most of the actinides in SYNROC would be incorporated in zirconolite. In nature, this mineral usually contains up to 1% of UO_2 and ThO_2 . Moreover, its structure is quite similar to that of pyrochlore, which commonly contains several per cent of $UO_2 + ThO_2$. Natural zirconolite and pyrochlore minerals are usually metamict because of the intense accumulated radiation damage from α particles. Nevertheless, they seem to have survived in their host rocks for periods exceeding 2,000 Myr without losing appreciable amounts of uranium and thorium. The temperatures, pressures and leaching conditions encountered by the natural minerals were probably far more severe than those of any proposed radwaste repository. The stability of these minerals in natural conditions and their demonstrable capacity to retain uranium and thorium provide grounds for confidence that synthetic zirconolite in SYNROC will be capable of immobilising the actinide elements for the required period of

~1 Myr, when buried in a carefully-chosen geological-geochemical environment.

In view of the the proved stability of 'hollandite' and perovskite in SYNROC B as demonstrated by their extreme resistance to leaching in experimental conditions, we can be confident that these minerals will be able to immobilise caesium and strontium for the required decay period of ~1,000 yr when SYNROC is buried in a suitable geological repository.

Operation of the SYNROC process

Our preferred process for immobilising radwaste is illustrated in Fig. 1. It is envisaged that high level waste (HLW) solution would continue to be flash-heated to produce 'calcine' as at present. SYNROC would be produced by a similar process from an aqueous solution-suspension containing the components in their correct proportions. Oxide mixtures produced by this procedure possess a high degree of reactivity and homogeneity.

About 90% of SYNROC would then be intimately mixed with ~10% of HLW calcine. The mixture would be introduced into a thick-walled cylindrical nickel container and uniaxially cold-pressed to ~70% of its theoretical density. A thick nickel lid would then be placed on the cylinder, separated by a thin copper seal.

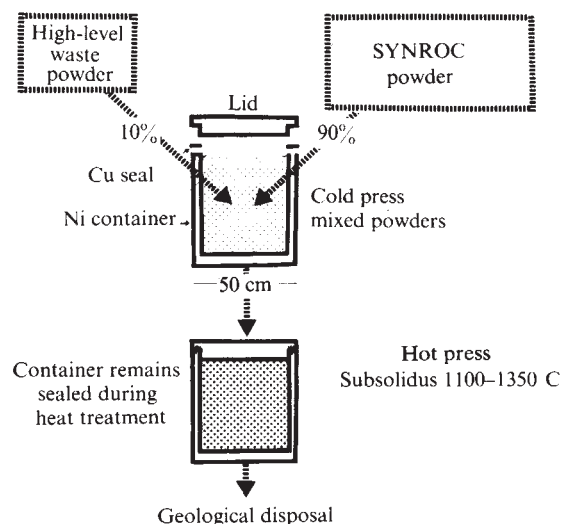


Fig. 1 Flowsheet for the immobilisation of radwaste in SYNROC and sealing of the resultant material in thick-walled nickel canisters.

The entire assembly would then be hot-pressed, either uniaxially or isostatically, at a temperature of 1,200–1,300 °C. In these conditions, rapid and complete subsolidus equilibration and recrystallisation of HLW calcine and SYNROC components occur, leading to the formation of a well-crystallised SYNROC mineral assemblage containing the HLW calcine components in solid solution. The mineral assemblage is mechanically strong and close to theoretical density.

During hot-pressing, the nickel container is mobile and flows inwards as the volume of the SYNROC contracts. The lid is sealed on to the container simultaneously by the copper, which melts at a lower temperature and alloys with nickel on either side. Two important advantages accrue from the above procedure. First, the formation of crystalline SYNROC in its terminal state and final encapsulation and sealing of the SYNROC in a metal capsule are performed in a single operation. Second, volatile components such as caesium and ruthenium are quantitatively retained by SYNROC minerals during the hot-pressing operation, and become permanently incorporated in the lattices of SYNROC minerals as described earlier. Radioactive contamination of the production plant and of the environment is thereby minimised. In contrast, during the production of borosilicate glasses, substantial amounts of caesium, ruthenium and other volatile elements are lost by

volatilisation. The recovery of these elements for recycling is an expensive operation.

Conclusion

If our society is to take advantage of the potential benefits of nuclear power, there can be no excuse for any policy which does not use the most advanced technology to safeguard future generations from the attendant dangers of high level nuclear wastes. It can hardly be claimed that immobilisation of radwaste in borosilicate glasses, currently favoured by the nuclear power establishment, satisfies this requirement.

We have demonstrated that a form of synthetic rock, SYNROC, can be produced which is vastly superior to glass in its ability to immobilise radwaste for long periods in appropriate geological-geochemical environments. Because of the simplicity of the production technique, and the absence of a need to

recycle escaping volatiles such as Cs and Ru, the cost of producing SYNROC may be comparable to, or even less than, glass.

SYNROC is immune from devitrification and is composed of mutually compatible phases, which possess crystal structures identical to those of minerals which are known to have survived in geological environments at elevated pressures and temperatures for periods of 20–2,000 Myr and to have retained radioactive elements for these periods. This fact provides grounds for confidence that the far shorter immobilisation period required for radwaste can be attained in the much less extreme pressure-temperature conditions present in a suitable geological repository. This confidence is reinforced by direct accelerated leaching tests at elevated pressures and temperatures.

Note added in proof: Pudovkina *et al.*⁹ have since observed that the mineral zirkelite which occurs in the gem gravels of Sri Lanka and contains up to 20% (UO₂+ThO₂), possesses the zirconolite structure.

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Evidence for late Precambrian plate tectonics in West Africa

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In the Gourma and Iforas regions (Mali) rifting occurred around 800–850 Myr ago along the eastern margin of the West African craton with a triple point in Mali, the Gourma being interpreted as an aulacogen. Oceanic closure around 600 Myr led to a collision between the passive continental margin of the West African craton and an active continental margin to the east displaying island arc and marginal trough volcano-clastic assemblages bordering a deformed continental mass intruded by a high-level batholith. The suture is marked by a string of positive gravity anomalies corresponding to the emplacement of ultrabasic and basic rocks including perhaps ophiolites. East–West shortening was accompanied by the translation onto the West African craton foreland of nappes including internal nappes displaying high pressure–low temperature metamorphism assemblages.

A CONTROVERSIAL subject in orogenesis^{1,2} is whether plate tectonics had an important role in the Proterozoic^{3,4} or whether *in situ* ensialic models fit the facts^{5,6} in the best way. To seek an answer, attention has been focused on the Pan-African, the youngest and best preserved of Precambrian orogenic belts. In the past few years a detailed geological and geophysical investigation has been carried out on the critical Iforas–Gourma region in Mali^{7–10} (Fig. 1), an area of about 300,000 km², crossing the West African craton and the Pan-African mobile belt to the East. The results show convincing evidence of a Wilson cycle ending with collision between a passive continental margin and an active continental margin.

Pre-Pan-African rifting and the Gourma aulacogen

The West African craton, stable since 1,700 Myr, is partly covered by thin flat-lying cratonic sediments younger than 1,000 Myr (refs 11, 12), but along its eastern margin the Gourma basin is characterised by deep subsidence with an accumulation of over 8,000 m of sediments⁹ (Fig. 2). The observed sedimentary sequence with an early terrigenous clastic phase at the base (formations I and II), followed by differentiated carbonate deposits indicating lateral passage platform-slope-trough (formation III) and ending with prograde continental clastic sediments (formations IV and V) is typical of a passive margin¹³. Furthermore analysis of palaeocurrents in the overlying Bandiagara Sandstone Group shows a converging drainage pattern centred on the Gourma with transport from the south-west and west. The shape of the basin, as defined by the distribution of slope sedimentary facies (breccias, turbidites) in the carbonate sequence and by the gravity pattern, is that of a trough orientated WSW–ENE representing a gulf of subsidence within the West African craton, which here forms an embayment. It presents all the characteristics of a typical aulacogen¹⁴. A detailed sedimentological study will be given elsewhere. Gravimetrically it is marked by positive anomalies which can be traced westwards across the West African craton and this suggests a deep crustal heavy source¹⁵. We conclude that a major change in sedimentation occurred around 850–800 Myr ago with rifting along the eastern margin of the West African craton with a triple point in Mali: the Gourma is thought to be a failed arm which has evolved as an aulacogen, a comparable situation with that of the Benue trough with respect to the Gulf of Guinea in the Cretaceous⁴.

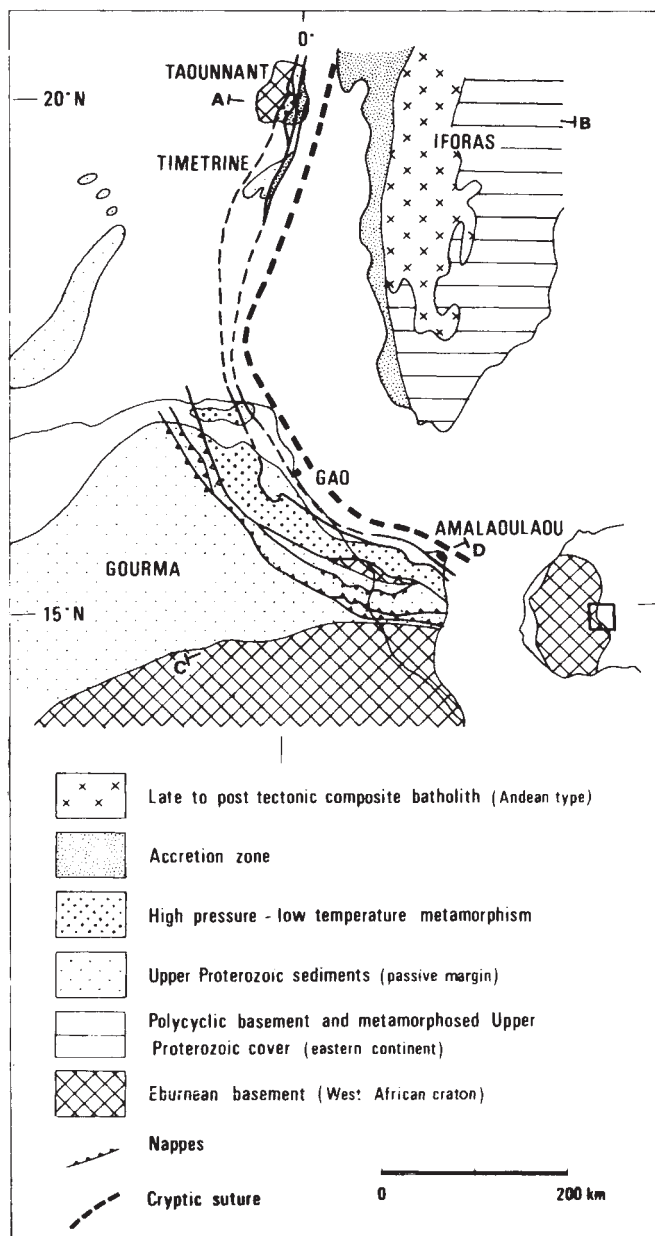


Fig. 1 Major structural units of the Iforas-Gourma region (Mali).

The associated magmatism would be represented to the North in northwestern Hoggar by the intrusion at around 800 Myr of gabbros and ultrabasic rocks into Upper Proterozoic quartzites and dolomites of shelf type^{12,16}, and by the presence of deformed north-south dyke swarms of alkaline and peralkaline metarhyolites, undersaturated soda-trachytes and metaphonolites superimposed on basic dyke swarms which may represent the roots of a pre-Pan-African palaeorift¹⁶. In northeastern

Gourma at Amalaoulaou gabbros and ultrabasic rocks are believed to have intruded the base of the crust at ~850 Myr before high pressure-high temperature granulite conditions and to have been tectonically emplaced around 600 Myr at a high level in the suture zone (Lancelot and de la Boisse, personal communication).

The suture

Oceanic closure 200 Myr later during the Pan-African is marked by a suture outlined by a string of positive gravity anomalies with amplitudes of over 30 mgls locally attaining 80 mgls which may be followed over a distance of 2,000 km and which correspond in the Iforas-Gourma region to the position of basic and ultrabasic complexes (mainly layered metagabbros and quartz gabbros) and may include ophiolites (Fig. 3). Interpretation of profiles across the anomalies¹⁵ using the inverse approach¹⁸ and linear programming¹⁹ shows that the structures, considered as homogeneous bodies with densities $>2.8 \text{ g cm}^{-3}$ and generally $>2.9 \text{ g cm}^{-3}$ continue to depths varying from 6 to 20 km. Geometry shows that bodies with a density of 3 g cm^{-3} must be unrooted. In shape they generally show an easterly dip which is in accord with the general movement pattern with thrusting towards the West African craton and with direct field observations made along the banks of the Niger.

South-west and west of the suture

In the Gourma (Figs 1, 4) nappes outcrop over a zone 300 km by 50–80 km wide²⁰. The internal nappes (mainly micaschists and quartzites) characterised by high pressure-low temperature metamorphism display flat-lying foliation. Foliation planes bearing phengite cut earlier sharp folds and the grade of metamorphism increases to the south-west where it attains eclogite conditions with a very pure jadeitic pyroxene (de la Boisse, personal communication). Stretch lineation association with NE-SW minor folds perpendicular to the general strike, are well developed within the nappes and along the lower grade mylonitic quartzite soles of the nappes. In northern Gourma the internal nappes come into direct contact with the para-autochthonous folded greenschist facies Gourma formations by underthrusting towards the south-west; to the south they are faulted against the Bourré Massif. The Bourré Massif²¹ is a sub-vertical horst of Eburnean granite dated at $2,080 \pm 20 \text{ Myr}$ ²² intrusive into subsequently retrograded amphibolite facies Birrimian sediments, overlain in unconformity by upper Proterozoic sericite quartzites and basal polygenic conglomerate. The horst was extruded after the passage of the external nappes, a situation comparable to that of the Mont Blanc in the western Alps. The external nappes are sub-horizontal and probably pellicular. They consist essentially of schistose formations belonging to the passive margin displaying greenschist facies metamorphism devoid of high pressure conditions. Over large areas one observes an upright succession and flat schistosity and a-type lineations within the nappes. The Gourma formations I–V described above¹³ as forming a pre-Pan-African aulacogen have been strongly deformed to form the para-autochthonous foreland. Major folds are overturned to the south-west and are superimposed on early pre-nappe east-

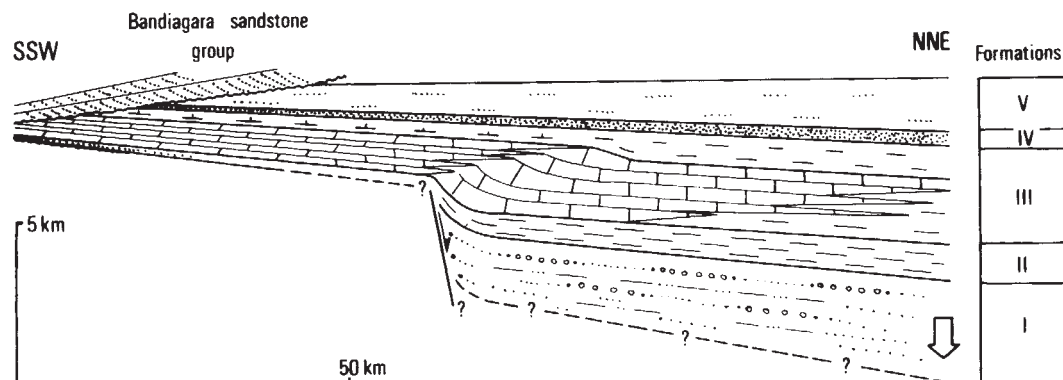


Fig. 2 Schematic SSW-NNE section showing variations in facies and thickness of the Gourma formations I–V.

west folds⁹. Between the front of the nappes and Hombori (100 km) several tens of kilometres of shortening implies a 'décollement' with respect to the underlying basement. The non-metamorphic Bandiagara Sandstone Group overlies in unconformity the Gourma Formations I–V and has been affected by a later phase of folding.

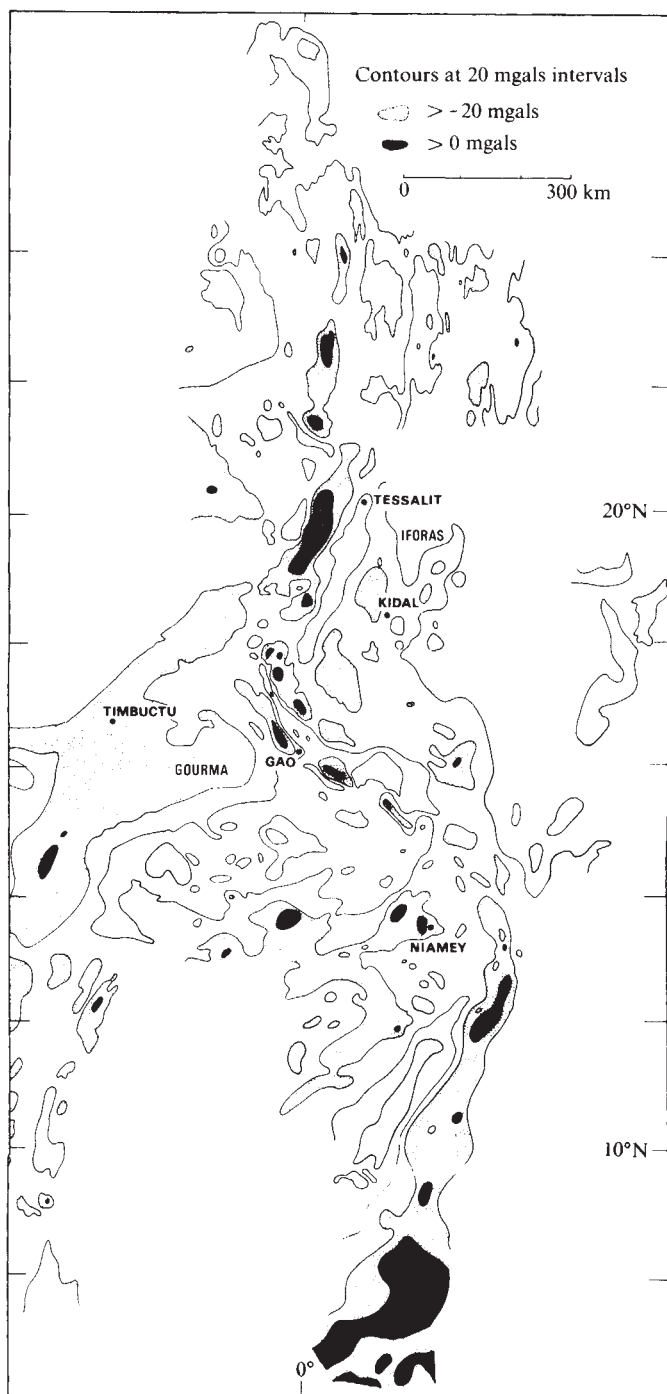


Fig. 3 Simplified Bouguer anomaly map of the eastern margin of the West African craton.

Four-hundred kilometres further north, in the Taounant region two flat-lying superimposed nappes have been mapped directly overlying eroded Eburnean (2,000 Myr old) basement. The lower one consists of a mylonitised unit of quartzites–dolomites presumably belonging to the passive continental margin whereas the overlying one is composed of strongly epidotised metabasalts and diabbases containing a blue amphi-

bole, which are thought to come from an oceanic domain to the east. Similarly to the South in the Timetrine, the quartzites, meta-arkoses, phyllites, large serpentine bodies and metabasalts with blue amphibole, are also interpreted as nappes, but here the relationships with the Eburnean basement are obscured by the Cretaceous.

A characteristic feature west of the suture is the total absence of autochthonous Pan-African magmatism. To the east of the suture a strongly deformed zone ~100 km wide is composed of a late Upper Proterozoic volcano–clastic assemblage. It consists of flysch-like metagreywackes with turbidites, conglomerates composed exclusively of volcanic and plutonic pebbles, dacitic breccias and meta-basalts and later meta-andesites volcanic greywackes and a terrigenous flysch unit, suggesting island arc and marginal sea environments, still active during the earlier deformation of the more internal continental domain of the Iforas, a situation similar to that of the upper Cretaceous to Mid-Tertiary flyschs of Western Alps. This assemblage is comparable to that of the 'Série verte' described 300 km further north in North-west Hoggar¹⁶ where geochemical studies have shown the immature nature of the greywackes²³ and the probable derivation of andesites in liaison with a subduction zone by partial melting of the mantle followed by low pressure fractionation²⁴. This zone is injected by a considerable volume of predominant pre-tectonic ultrabasic rocks, gabbros and diorites the volume of more acid terms, tonalites and adamellites increasing eastwards. This accretion zone, apparently devoid of an ancient sialic substratum, follows the sheared western margin of the Iforas. It is significant that greywacke formations with associated calc-alkaline magmatism which are widely developed in western Hoggar and the Iforas are totally absent west of the suture. Such an absence would be explained by the former existence of an ocean separating two continents.

The Iforas is a highly deformed sheared continental domain of 2,000 Myr old granulite basement with remnants of supracrustal platform deposits (quartzites and dolomites) overlain by late Upper Proterozoic marginal sea volcano–clastic sediments including a possible tillite intruded by abundant pre-, syn- and post-tectonic granitoids. A zone of late high temperature–low pressure metamorphism with the local occurrence of granulite facies rocks, charnockites and norites (Aguelhoc, Iforas, Egatalis, North-west Hoggar) coincides with the westernmost known area of sialic rock and its Upper Proterozoic cover²⁰. A vast composite late to post-tectonic calc-alkaline batholith capped by flat-lying rhyolitic lavas (Nigritian)¹⁰ and cut by sub-alkaline and alkaline ring-complexes occupies the external zone of the continental domain. It coincides with a regional positive gravity anomaly. Emplacement occurred in a zone subjected to pronounced vertical uplift leading to unroofing during the Pan-African. Existing U–Pb data on Zircons (Lancelot, personal communication) suggest this late batholith to have been emplaced in a relatively short interval between 615 and 590 Myr, the dates 616 ± 11 Myr and 613 ± 3 Myr corresponding respectively to a diorite and quartz monzonite, and 586 ± 13 Myr to a hedenbergite–hastingsite–perthite granite belonging to the Kidal ring-complex. Magmatic activity in this zone, however, must have taken place over a long period of time, as the late batholith cuts foliated diorites corresponding to a deeply eroded pre-tectonic palaeobatholith about 700 Myr old. A striking feature of the batholith is the presence of spectacular dyke swarms. Early east–west striking swarms consist essentially of subordinate basic dykes, quartz-microdiorites micro-adamellites and felsites which locally were truncated by high-level adamellite and perthite granite. They are cut by important north–south swarms composed of subordinate basic dykes and several generations of quartz microsyenite, granophyres and rhyolites which can be followed over a distance of over 250 km in the axis of the batholith and which coincide with the Nigritian rhyolite fields and the alignment of ring-complexes. In a different context the batholith displays features reminiscent of an Andean-type batholith²⁵. The general disposition of calc-alkaline magmatism east of the suture suggests an easterly

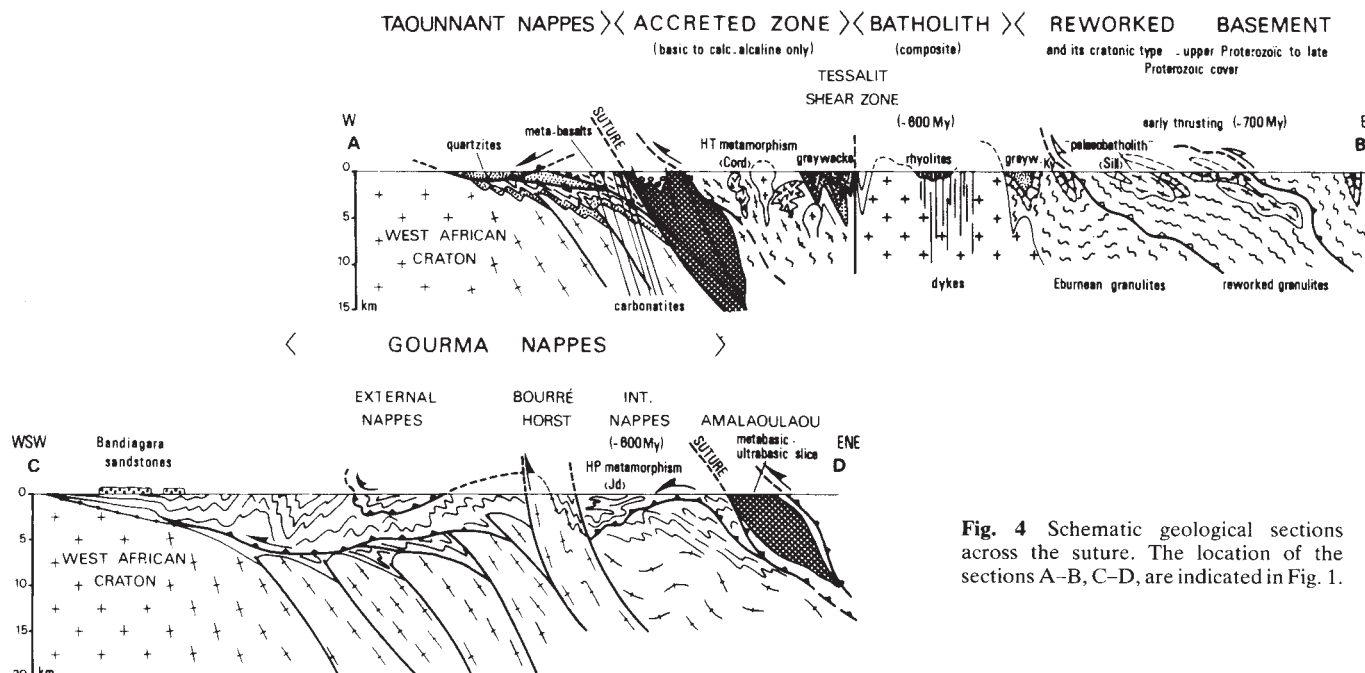


Fig. 4 Schematic geological sections across the suture. The location of the sections A-B, C-D, are indicated in Fig. 1.

dipping palaeo-subduction zone and the high pressure-low temperature metamorphism of the internal nappes of the Gourma would fit with this picture. The late alkaline rocks in the ring-complexes may be related to verticalisation of the Benioff plane accompanied by a diapiric rise of mantle material east of the suture²⁶.

General movement pattern

The Eburnean basement and supracrustal Upper Proterozoic formations of the Iforas have a complex structural and metamorphic history. Early Pan-African intracontinental deformation dated by the earliest syntectonic granites at 693 ± 0.3 Myr (Renaud Andreopoulos, personal communication) and which took place in barrowian metamorphic conditions generated large northwesterly moving crystalline nappes which were subsequently refolded, leading to considerable tectonic thickening and locally pronounced NW-SE metamorphic gradients²⁷. This early phase of deformation produced ENE-WSW structures which have also been observed in the accretion zone and suggest an early collision still not well defined. Late Pan-African deformation directly related to collision with the West African craton resulted in considerable east-west shortening in western Iforas and in the translation of nappes onto the foreland west and south-west of the suture. A characteristic feature east of the suture is the spectacular development of shear belts with a complex history. There is evidence for early sinistral movement along N° 20 trending shear belts and in the closing stages of collision for dextral movement on north-south belts leading to the northwards displacement of western and central Iforas with respect to eastern Iforas. Molassic sediments similar to the 'Série Pourprée' of western Hoggar dated at around 530 Myr (ref. 7) occur in grabens and fill a north-south palaeo-rift on the West-African craton between the suture and Timetrine-Taounant where it is associated with under-saturated syenites and carbonatites. Late movements as young as the Upper Cambrian produced open north-south folds in the molassic sediments and a system of conjugated NNW sinistral and ENE dextral wrench faults, a system largely developed throughout the Pan-African mobile zone of West Africa²⁸.

Conclusions

All the evidence points to a modern-type orogenic belt involving a collision during the closing stages of the Pan-African, between the passive continental margin of the West African craton and

the active continental margin of an eastern continent. This conclusion fits the recognition of obducted ophiolites at Bou Azzer (Morocco) along the northern margin of the West African craton²⁹ and supports the collision hypothesis advanced for the Togo-Benin segment³. Looking at the Touareg shield (Hoggar, Air, Iforas) as a whole, the general pattern of shear belts and late Pan-African deformation seen at an eroded level, shows striking analogies with the tectonic pattern of Asia produced by the collision of India³⁰. Palaeomagnetic evidence suggests important horizontal displacement during the Pan-African³¹, and proposed reconstructions for 800 Myr and 675 Myr indicate open situations between North America and Gondwanaland and oceanic closure at 600 Myr (refs 32, 33). As no measurements exist for the West African craton, these results are not incompatible with the former presence of a pre-Pan-African ocean situated east of the West African craton.

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Structure of the human fetal globin gene locus

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We have derived a 'map' of restriction enzyme sites in and around the human γ -globin genes. This has enabled us to show that there are two γ -globin genes per haploid set, that the genes contain 'introns' within the same regions of DNA as the human β and δ -globin genes, and that the genes are 3,500 base pairs apart. We conclude that the correct gene organisation of the human β -like globin locus is $^G\gamma^A\gamma\delta\beta$.

DURING human fetal life, the major haemoglobin is HbF, consisting of two α -globin and two γ -globin chains ($\alpha_2\gamma_2$). In the last stages of fetal development, a switch occurs from γ -globin synthesis to adult β -globin synthesis; by a few weeks after birth, the major haemoglobin is HbA ($\alpha_2\beta_2$) accompanied by a much lower level of HbA₂ ($\alpha_2\delta_2$)¹. In some populations (such as Saudi Arabians), in most persons at times of erythropoietic stress (as in severe anaemia, β -thalassaemia, or sickle cell disease) and in pregnancy, the proportion of HbF in adult peripheral blood rises. In addition, in the rare condition known as hereditary persistence of fetal haemoglobin (HPFH) there is no HbA or HbA₂ and the person, who is clinically asymptomatic, survives solely on HbF (refs 2, 3).

It is known that there are at least two non-allelic γ -globin genes, one of which codes for a γ -globin chain with glycine and the other with alanine at amino acid position 136 of the 146 amino acid γ -chain ($^G\gamma$ and $^A\gamma$ respectively)⁴. In people with a mutation at some other position, either the $^G\gamma$ or the $^A\gamma$ -globin chain shows the mutation, but never both. It can be concluded that there must be at least two γ -globin genes per haploid genome, one each for the $^G\gamma$ and $^A\gamma$ -globin chains. Molecular hybridisation to cellular DNA of γ -globin complementary DNA supports this conclusion^{5–7}.

However, the chain synthesis ratios of $^A\gamma$ and $^G\gamma$ are not those predicted if protein synthesis reflects gene number, and therefore it has been suggested that there are three or four γ -globin genes per haploid genome⁸. It has also been suggested that a third γ -globin gene may occur, so-called $^T\gamma$, coding for HbF^{sardinia}, with threonine rather than isoleucine in position 75 (ref. 9). $^T\gamma$ expression varies greatly among individuals and may represent either a third non-allelic γ -globin gene, or a commonly occurring allele of one of the existing genes. It is not yet possible to distinguish genetically between these alternatives.

We have recently reported the physical map of the human δ and β -globin gene loci¹⁰ as have others¹¹. Here we describe similar experiments which allow the mapping of the human γ -globin genes within their surrounding DNA sequences.

The development of techniques for the mapping of single-copy genes^{12–15} makes it possible to construct a physical map of restriction enzyme sites in and around the γ -globin genes. Normal human placental DNA is digested with restriction

enzymes and denatured. The fragments are separated by electrophoresis on a 1.2% agarose gel, transferred from the gel to a nitrocellulose filter using the method of Southern¹² and hybridised with ³²P-labelled pH γ G1 DNA followed by autoradiography to reveal the position of the fragment containing γ -globin gene sequences. pH γ G1 is a pCR1-derived recombinant that contains 500 base pairs of double-strand complementary DNA prepared from human $^G\gamma$ -globin mRNA¹⁶. The limitations of this technique have been extensively discussed^{10,12,14,15}.

Our approach to mapping the γ -gene locus has been to identify a restriction endonuclease which gives only a single fragment containing all the γ -globin gene sequences hybridising to the specific γ -globin recombinant probe, to locate the gene sequences within this fragment by making use of restriction enzymes which are known to cleave within the globin coding sequence, and to distinguish between the several (in this case, two) gene sequences by identifying a restriction site present in one and absent in the other.

Table 1 Summary of sizes of γ -globin gene fragments generated by a variety of restriction enzymes in single and double digests

<i>Bgl</i> II	<i>Bam</i> HI	<i>Eco</i> RI	<i>Pst</i> I	<i>Xba</i> I	<i>Bgl</i> II+ <i>Bam</i> HI	<i>Bgl</i> II+ <i>Eco</i> RI
13	15.0	6.5	10.0	7.5	6.0	3.0
	5.0	2.5	5.0	5.0	5.0	2.5
	2.6	1.65	4.0	3.7	2.1	1.65
		0.65	0.9			0.65
<i>Bgl</i> II+ <i>Pst</i> I	<i>Bgl</i> II+ <i>Xba</i> I	<i>Bam</i> HI+ <i>Eco</i> RI	<i>Bam</i> HI+ <i>Xba</i> I	<i>Eco</i> RI+ <i>Pst</i> I	<i>Pst</i> I+ <i>Xba</i> I	
5.0	6.0	2.6	7.5	4.0	5.0	
2.2	5.0	1.65	5.0	1.8	3.7	
0.9	2.25	1.05	2.6	1.6	0.9	
		0.65		0.8		

Sizes are in kilobase pairs.

High molecular weight DNA was isolated from placentae of three normal infants of English, Welsh and Dutch ancestry. The DNA was cleaved with the restriction enzyme *Bgl*II, and after electrophoresis, transfer and hybridisation to ³²P-labelled pH γ G1 DNA only a single fragment of about 13 kilobases is seen. This fragment contains all the γ -globin gene sequences in human DNA. (All γ -globin gene fragment sizes are presented in Table 1.)

Several restriction endonucleases are known to cleave the γ -globin gene sequence from direct analysis of plasmid recombinants constructed with cDNA prepared from γ -globin mRNA^{16–18}. Table 2 lists these enzymes, together with the corresponding amino acid positions coded for at the cleavage site. *Bam*HI and *Eco*RI cleave both the $^A\gamma$ and the $^G\gamma$ genes, in

Table 2 Restriction enzyme sites within the γ or γ coding sequence

	A γ	G γ
<i>EcoRI</i>	121–122	121–122
<i>BamHI</i>	99–100	99–100
<i>PstI</i>	135–136–137	None
<i>XbaI</i>	None	None
<i>BglII</i>	None	None

Sites given are for the amino acids coded for by the restriction site in the appropriate γ -globin gene. Numbering is from the N(5') terminus of the protein chain. The positions of the *EcoRI* and *BamHI* sites are from refs 16 and 17; the *PstI* site from ref. 18, and the absence of *XbaI* and *BglII* sites from our unpublished data.

the same coding positions as for the human β and δ -globin genes, whereas *PstI* cleaves only the γ gene and not the γ (ref. 18). It is known that the coding sequence for γ contains a *PstI* site at the position coding for amino acids 135–136–137 (–CTGCAG–). This site is absent from the γ gene because of the presence of glycine rather than alanine at this position of the amino acid chain, giving a sequence altered to (–CTGGAG–) which is not cleaved.

When normal DNA is cleaved with *EcoRI*, four fragments hybridising to the γ -globin probe are obtained, of sizes 6.5, 2.5, 1.65 and 0.65 kilobases (Fig. 1). Assuming at this stage that there are two genes, this is consistent with the presence of at least one additional *EcoRI* site between the two genes. If this prediction is valid, these four fragments include two derived from the 3' end of the genes and two derived from the 5' end.

A double digest with *BglII* and *EcoRI* again gives four fragments (Fig. 1), three of which (at 2.5, 1.65 and 0.65 kilobases) are identical to those from an *EcoRI* digest and one of which, of 3.0 kilobases, is new and replaces the band of 6.5 kilobases, from which it must be derived. (It is too large to be

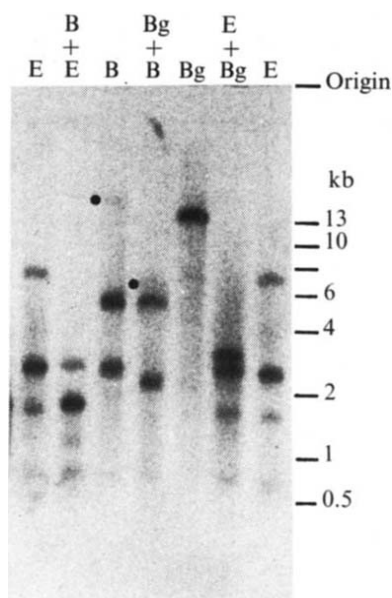


Fig. 1 γ -Globin gene containing fragments generated by *EcoRI* (E), *BamHI* (B), *BglII* (Bg) and combinations of these enzymes. The calibration scale is in kilobase pairs and faint bands are marked by ●. 30 μ g of placental DNA, prepared according to ref. 10, was digested with the appropriate restriction endonuclease, denatured with alkali and electrophoresed on a 1.2% Agarose gel. The denatured DNA was transferred to a cellulose nitrate filter sheet by blotting¹², the filter cut into strips and hybridised with 50 ng per ml of ³²P-labelled pH γ G1, at 65 °C in 3 $\times$ SSC 0.1% SDS, for 2 d (SSC is 0.15 M NaCl, 0.015 M Na citrate pH 7.0). After hybridisation, strips were washed with 3 $\times$ SSC 0.1% SDS at 65 °C and finally with 0.1 $\times$ SSC 0.1% SDS also at 65 °C. Labelled components were then detected by autoradiography. All details of hybridisation and washing procedures and of the size markers used are as described in refs 14 and 15.

generated from any other *EcoRI* fragment). Therefore an *EcoRI* site internal to one of the γ -globin genes must be located 3.0 kilobases from one of the bounding *BglII* sites, and a second *EcoRI* site must lie 3.5 kilobases to the other side of this *BglII* site. The three smallest *EcoRI* fragments are not cleaved by *BglII* and therefore must lie entirely within the large 13-kilobase *BglII* single digest fragment.

BamHI single digests of human DNA give three hybridising fragments of 15, 5.0 and 2.6 kilobases (Fig. 1). As both genes contain a *BamHI* site, these three fragments could result from the *BamHI* cleavage of each gene, with no additional *BamHI* site in the DNA sequence between the two genes. The two *BamHI* sites are localised within the 13-kilobase *BglII* fragment, as shown using a double digest of *BamHI* plus *BglII*. The 15.0-kilobase *BamHI* fragment is cleaved by *BglII* to give a 6.0-kilobase double digest fragment; the 2.6-kilobase *BamHI* is cleaved by *BglII* to give a 2.1-kilobase double digest fragment (Fig. 1). The 5.0-kilobase *BamHI* fragment is not cleaved by *BglII*. Thus, *BamHI* sites are located 6.0 and 2.1 kilobases, respectively, from the bounding *BglII* sites of the 13-kilobase *BglII* fragment. Other *BamHI* sites are 15.0 and 2.6 kilobases, respectively, outside these internal *BamHI* sites, as defined by the *BamHI* single digest fragments.

From these data, the distance predicted between the two *BamHI* sites within the 13-kilobase *BglII* fragment is 4.9 kilobases. We conclude that these *BamHI* sites generate the 5.0-kilobase fragment seen in the *BamHI* single digest, and that this fragment is sandwiched between the 15.0 and the 2.6 kilobase *BamHI* fragments (Fig. 2).

The 5.0-kilobase *BamHI* fragment has at its termini the only two *BamHI* sites which are present within the 13-kilobase *BglII* fragment. These must be the *BamHI* sites which are located within the two γ -globin genes (Table 2). The 5.0-kilobase *BamHI* fragment contains two 'half-genes' therefore (one to the 3' side and one to the 5' side of the intragenic *BamHI* site), and each of the other *BamHI* fragments contains one 'half-gene'.

It is possible to orientate the *BamHI* and *EcoRI* 'maps' with respect to the *BglII* sites by use of a *BamHI*/*EcoRI* double digest. We have shown that both the 2.6-kilobase *BamHI* and the 6.5-kilobase *EcoRI* fragments contain a *BglII* site. There are two possibilities: either the *BglII* site is the same in both the *EcoRI* and the *BamHI* fragments (as shown in Fig. 2), which predicts that the 6.5-kilobase *EcoRI* fragment will be cut by *BamHI* to yield a 2.6-kilobase double digest fragment. Alternatively, the 6.5-kilobase *EcoRI* and the 2.6-kilobase *BamHI* fragments are located at opposite ends of the 13-kilobase *BglII* fragment. In this case there would be no *BamHI* cut in the 6.5-kilobase *EcoRI* fragment (Fig. 2). The fact that a double digest with *BamHI*/*EcoRI* gives fragments of 2.6, 1.65, 1.05 and 0.65 kilobases, eliminates this second possibility.

The existence of four *EcoRI* fragments is compatible with two γ -globin genes. The *BglII*/*EcoRI* digest above locates an *EcoRI* site within one of these. The *BamHI* plus *EcoRI* digest locates the *EcoRI* site in the other γ -globin gene, and establishes that there are two γ -globin genes. The strongly hybridising 2.5-kilobase *EcoRI* fragment is cleaved by *BamHI* to give a 1.65-kilobase double digest fragment (Fig. 1); alternative possibilities are inconsistent with a *PstI*/*EcoRI* double digest (Fig. 3a).

The orientation of the 2.5-kilobase *EcoRI* fragment with respect to the *BamHI* sites is established by experiments with 3'- and 5'-specific probes (data not presented; see Fig. 4 legend). The 15-kilobase *BamHI* fragment does not hybridise with 5'-specific probes, whereas the 5.0-kilobase *BamHI* fragment and the 2.5-kilobase *EcoRI* fragment do. Hence the orientation of the 2.5-kilobase *EcoRI* fragment can only be that shown in Fig. 2. According to the *BamHI*-*EcoRI* map derived from these data, the 1.65-kilobase *EcoRI* fragment would be uncut by *BamHI* and would co-migrate with the 1.65-kilobase *BamHI* + *EcoRI* double digest fragment.

Therefore, the intragenic *BamHI* and *EcoRI* sites in both coding sequences are 800–1,000 base pairs apart. From the direct sequence known from the cDNA plasmids, it is known

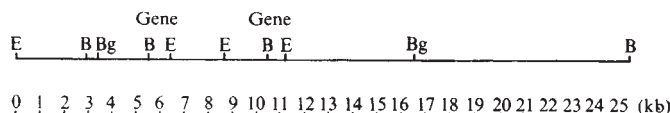


Fig. 2 Partial restriction site map of the γ -globin genes. Only approximate positions of the structural genes are shown. E, *EcoRI*; B, *BamHI*; Bg, *BglII*.

that the corresponding sites in the mRNAs are only 67 base pairs apart. This difference defines an intervening sequence (intron) between the *EcoRI* and the *BamHI* sites, at the same positions as those previously demonstrated in the rabbit and mouse β -globin genes and in the human δ and β -globin genes (refs 10, 11, 15, 19 and T. Maniatis, personal communication). The 1.05-kilobase *EcoRI*+*BamHI* double digest fragment is presumably generated from the intragenic *BamHI* and *EcoRI* sites flanking the large intron—the size of this fragment is in reasonable agreement with that predicted (0.85) from the map position of the *EcoRI* and *BamHI* sites described above. Figure 2 shows the map of the *BglII*, *BamHI* and *EcoRI* sites located by these experiments. (We will refer to the γ -globin genes as 'left' and 'right' as displayed in this figure.)

Cleavage of human DNA with *XbaI* gives three γ -globin gene fragments of 7.5, 5.0 and 3.7 kilobases (Fig. 3). A double digest with *BglII* yields fragments of 6.0, 5.0 and 2.25 kilobases. Thus, the 3.7-kilobase *XbaI* fragment is cleaved by *BglII*: this places an *XbaI* site 2.25 kilobases from the *BglII* site and a second *XbaI* site 3.7 kilobases away from this *XbaI* site. The 7.5-kilobase *XbaI* fragment is cleaved by *BglII* to give the 6.0-kilobase double digest fragment and this places an *XbaI* site 6.0 kilobases from the second *BglII* site. To orientate the *XbaI* sites with respect to the *BamHI* sites and within the *BglII* fragment, *XbaI* plus *BamHI* double digests were carried out. This yields the 7.5-kilobase *XbaI* fragment, the 5.0-kilobase fragment present in both *BamHI* and *XbaI* digests and the 2.6-kilobase *BamHI* fragment (Fig. 3). As the 2.6-kilobase *BamHI* fragment is generated from the 3.7-kilobase *XbaI* fragment, this allows the orientation of the *XbaI* sites with respect to the *BglII* sites. The only possible explanation for the existence of the 5.0-kilobase *BamHI* plus *XbaI* double digest fragment is that the two *XbaI* sites within the *BglII* fragment are located very close to the

position of the two *BamHI* sites mapped above. As *XbaI* does not cleave the γ -globin cDNA, and as the *BamHI* sites considered here are within the coding sequence of both γ -globin genes, the *XbaI* sites must be present in the intron, between the *BamHI* and *EcoRI* sites in both γ genes.

PstI generates γ -globin gene fragments of 5.0, 4.0 and 0.9 kilobases and a faint band of about 10 kilobases (Fig. 3). The *PstI* sites have been mapped in a double digest of *PstI* plus *BglII*, *EcoRI* or *XbaI*. Cleavage of human DNA with *PstI* plus *BglII* gives γ -gene fragments of 5.0 and 0.9 kilobases (present in the *PstI* digest) and a double digest fragment of 2.2 kilobases (Fig. 3). This fragment must be derived from the 4.0-kilobase *PstI* fragment by *BglII* cleavage which places a *PstI* site 2.2 kilobases from a *BglII* site and also a *PstI* site 4.0 kilobases away from this *PstI* site. The *PstI* and *XbaI* double digest orientates the *PstI* sites within the *BglII* fragment: fragments of 5.0, 3.7 and 0.9 kilobases are obtained (Fig. 3) showing that the 4-kilobase *PstI* fragment contains the 3.7-kilobase *XbaI* fragment. A 5.0-kilobase fragment is present in both *PstI*, *XbaI* and *PstI*+*XbaI* digests which suggests that the *PstI* and *XbaI* sites which generate these 5-kilobase fragments must map near to each other. This is confirmed by the fact that one of the *PstI* sites, located above using *PstI* plus *BglII* double digests, maps very close to an *XbaI* site, independently located using a double digest with *BglII*, which defines one end of the 5.0-kilobase *XbaI* fragment. The 4.0 and 5.0 kilobase fragments are therefore contiguous on the DNA at a single *PstI* site. The position of the 0.9-kilobase *PstI* fragment will be discussed below.

Orientation of the physical map

To orientate the physical map with respect to the direction of transcription it is necessary to determine the orientation of the structural gene sequence. We have done this by hybridising restriction endonuclease digests to separate probes for the DNA regions to the 5' or 3' side of the intragenic *EcoRI* site. Plasmid pHYG1 was cut with a mixture of *EcoRI* and *HindIII* restriction endonucleases: this gives two fragments of about 4 and 10 kilobases containing the γ -globin gene sequence to the 3' and 5' side of the *EcoRI* site, respectively. The two fragments were purified by gel electrophoresis, recovered from the gel²⁰ and labelled with ³²P by nick translation.

Filters carrying *EcoRI*-digested human DNA were hybridised with 3'- and 5'-specific probes. The 5' probe detected only the 6.5 and 2.5 kilobase fragments whereas the 3' probe detected the 1.6 and 0.65 kilobase fragments (Fig. 4).

Similar experiments (not presented) indicated that the 15-kilobase *BamHI* fragment contains only 3' information. This, combined with the fact that the 2.5-kilobase *EcoRI* fragment is only 5' containing, indicates that the orientation of the 2.5-kilobase *EcoRI* site with respect to the *BamHI* site in the right hand γ -gene can only be that shown in Fig. 2.

Localisation of the 3' *EcoRI* fragments

The mapping data described above do not allow us to locate the 1.65 and 0.65 kilobase *EcoRI* fragments. As both fragments contain DNA sequences to the 3' side of the *EcoRI* site in the γ -globin genes we anticipated that one fragment must be derived from the γ gene, and one from the α gene. To assign the *EcoRI* fragments to the γ genes we performed a *PstI* and *EcoRI* double digest. The α gene has a *PstI* site at position 136 and *PstI* should therefore cut the 3' *EcoRI* fragment of this gene whereas the γ gene has no such site. Strongly hybridising fragments of 4.0 and 1.8 kilobases, together with a fainter band at 1.6 kilobases were seen in this digest (Fig. 3). The 4.0 and 1.8 kilobase bands contain the 5' regions of the gene to the left and right in the map in Fig. 2, up to the *PstI* sites in each respective intron. The size of these fragments fits exactly with the map deduced in the preceding sections. The 1.6-kilobase band is the size expected for the 3' *EcoRI* fragment uncut by *PstI*. This fragment therefore must lie within the 5-kilobase *PstI* fragment and adjoin the 6.5-kilobase *EcoRI* fragment of the left hand

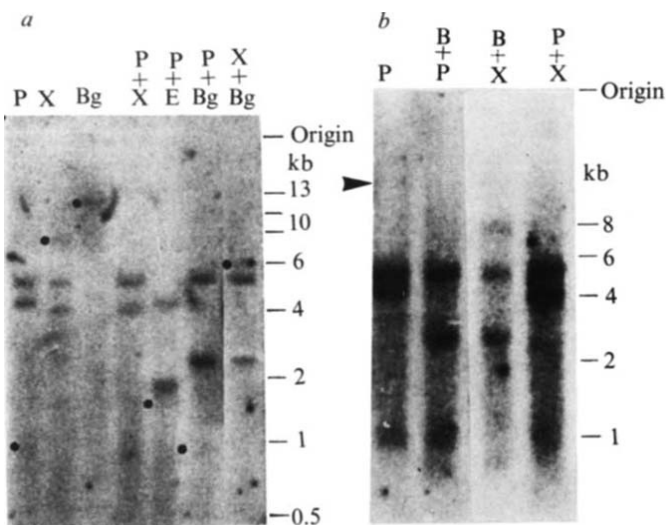


Fig. 3 *a*, γ -globin gene containing fragments generated by *PstI* (P), *XbaI* (X), *BglII* (Bg) and *EcoRI* (E) in single and double digests. Sizes are in kilobases. The position of certain faint bands is marked ●. *b*, γ -globin gene containing fragments generated by *PstI* (P), *XbaI* (X) and *BamHI* (B) in selected single and double digests. Sizes are in kilobases. The arrow marks the position of the very faint 10-kilobase *PstI* fragment seen in some digests. Experimental conditions for both experiments are as in Fig. 1.

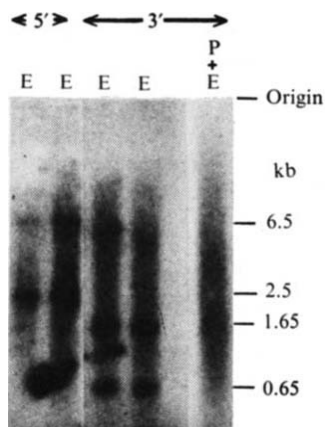


Fig. 4 The orientation of the γ -globin genes within the neighbouring DNA. 30 μ g of *EcoRI* digested DNA (E) or 30 μ g of *EcoRI*+*PstI* digested DNA (E+P) was alkali denatured and run on 1.2% Agarose gels and transferred to filters (as described in Fig. 1). Strips were then hybridised with 32 P-labelled 4-kilobase pH γ G1 *EcoRI*+*HindIII* fragment (3' specific) and the 10-kilobase *EcoRI*+*HindIII* fragment (5' specific), as described in Fig. 1. In each case a threefold excess of unlabelled fragment of the opposite specificity is included in the hybridisation to reduce cross hybridisation due to probe contamination¹⁴. The running position of each size fragment is indicated.

γ -gene. The 0.65-kilobase *EcoRI* fragment cannot be seen clearly in the digest. The *PstI*/*EcoRI* digest was hybridised with a specific probe for the regions of the γ -gene to the 3' side of the intragenic *EcoRI* site (as described above in Fig. 4) to confirm these assignments. The 1.6-kilobase fragment is visible (Fig. 4) whereas the 0.65-kilobase fragment cannot be seen. (Compare with the *EcoRI* digests in Fig. 4 where both the 1.6-kilobase fragment and the 0.65-kilobase *EcoRI* fragment are clearly visible.) The 0.65-kilobase *EcoRI* fragment is cut by *PstI*, (presumably) at the region coding for amino acids 135–136–137 of the γ -globin polypeptide chain (see Table 1) which should remove 45 base pairs of DNA homologous to our probe. The remaining 100 base pairs should however be detected by the γ -probe. We have shown that this is the 3' end of the γ and the failure to detect this 100-base pair fragment may be the result of low homology between the 3' region of the γ gene and the γ cDNA probe in use here (data not presented). A similar problem arose in our study of the δ - β -locus¹⁰ where we could detect the 3' regions of the δ -globin gene with β -globin cDNA sequences only with difficulty.

Finally, the *PstI* cleavage of the 0.65-kilobase *EcoRI* fragment explains the 0.9-kilobase *PstI* fragment. This fragment is derived from the γ gene and extends from the *PstI* site in the intervening sequence of the gene to the *PstI* site at the DNA coding position for 136 of the γ -globin chain. We predict that a fourth *PstI* fragment containing the regions to the 3' side of the DNA coding for amino acid 137, should also be visible. This is seen as a very faint 10-kilobase fragment in some *PstI* digests (see Fig. 3, for example). As discussed above, we believe that this fragment is difficult to see for three reasons: it does not cross react well with our γ probe, it contains only 100 base pairs of DNA homologous to our probe and it is large.

The arguments presented here show that the leftward γ -gene in Fig. 2 is γ and the rightward gene is γ . We have not shown that the 3' *EcoRI* fragments are contiguous with their respective 5' fragment at the intragenic *EcoRI* sites of the γ genes. This could most simply be achieved by use of γ -globin chain mutants which have lost the *EcoRI* site, but these mutants are extremely rare^{1,2,22}. For the γ gene, non-contiguity of the 2.5 and the 0.65 kilobase *EcoRI* fragments implies that a second *PstI* site must occur exactly 0.9 kilobases from both the *PstI* site close to the *BamHI* site, and the intragenic *PstI* site. Similarly, the γ gene cannot contain more than an additional 0.75 kilobases of DNA, as this is all the DNA that is not included in gene-containing

fragments between the two γ -globin genes. We believe, therefore, that it is reasonable to assume contiguity of fragment round the intragenic *EcoRI* sites.

All the restriction site information derived above can be combined into the single map in Fig. 5a. We believe that this is the simplest map compatible with our data; other more complicated maps are not excluded, however. For example, the difficulty of detecting small fragments containing only short regions of structural genes makes it hard to eliminate the possibility that additional *EcoRI* sites are present (within an additional intron?) in the 3' regions of the two genes. We believe that this is unlikely for the same reasons discussed for the contiguity of the *EcoRI* fragments around the intragenic *EcoRI* sites. Molecular cloning of this locus will ultimately resolve this problem.

The major features of the map, shown in Fig. 5b, are as follows. We identify the γ gene by the presence of the *PstI* site to the 3' side of the intragenic *EcoRI* site. The γ gene is proved to be to the 3' side of the γ gene as predicted previously from genetic evidence^{3,22}. Both genes contain introns between the *BamHI* sites (at position coding for amino acids 99–100) and the *EcoRI* site (at positions 121–122). These introns are approximately 800–1,000 base pairs in length, similar to those found in the human δ - and β -globin genes. Similar sequences in the same position have previously been found in mouse¹⁹ and rabbit β -globin genes¹⁵. The two γ -globin genes are separated by 3.5 kilobases of DNA, as compared with approximately 7.0 kilobases of DNA separating the human δ - and β -globin genes¹⁰ and the map spans 25.5 kilobases of DNA.

Several more general conclusions can be drawn. Our data are consistent with the presence of only two γ -globin genes; for more to be present, a reduplication of an entire region of DNA greater than 25 kilobases in length and sufficiently conserved to retain identical restriction sites over this length would be

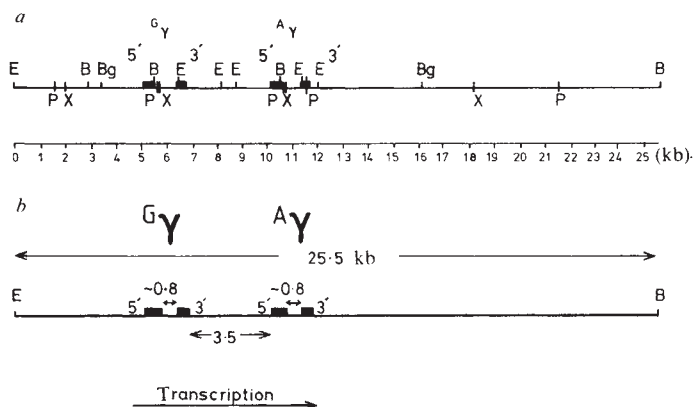


Fig. 5 a, Physical map of the linked γ and γ genes. The position of the coding sequences are indicated by filled boxes. It should be stressed that only those restriction sites that generate fragments containing a portion of coding sequence are detected in these experiments. The coding sequences are shown as two blocks. There is no evidence to exclude the possibility that these blocks might contain further short introns. E, *EcoRI*; B, *BamHI*; Bg, *BglII*; P, *PstI*; X, *XbaI*. b, The major features of the human fetal globin gene locus.

required. In the individuals examined (from persons of English, Dutch and Welsh parentage) there is no demonstrable third γ gene; therefore it is likely that the gene for HbF_{Sardinia} is an allele of one of the two γ -globin genes or that there is a polymorphism of γ -gene number in humans. We did not determine whether HbF_{Sardinia} was expressed in cord blood in these individuals and therefore final determination of the γ -globin gene must await investigation of a person who definitely synthesises this chain. In the small number of DNA samples used, no polymorphisms in the restriction fragment patterns were seen.

The demonstration of close linkage of γ and γ enables us to exclude one of the two models for the organisation of the

β -related globin gene family locus in humans. The existence of the fusion globins, Hb Lepore, where the N-terminal protein sequence of the δ -globin chain is fused to the C-terminal sequence of the β -globin chain²³, and Hb Kenya²⁵ where the N-terminal protein sequence of γ is fused to the C-terminal sequence of β -globin, had shown that the gene order must be either $\gamma\alpha\gamma\delta\beta$ or $\alpha\gamma\delta\beta\gamma$. We have demonstrated that the former is correct.

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letters

Infrared observations of the X-ray quasars 0241+622 and MR2251-178

TWO new quasars have recently been found through X-ray observations (the only other one being 3C273, see refs 1, 2). Ricker *et al.*³ have reported observations confirming the identification of the X-ray source 2251-178 with a quasar of visual magnitude 14.1. Canizares, McClintock, and Ricker⁴ have reported visual spectrophotometric observations of this quasar, and find its visual spectrum is much like other low redshift quasars. Apparao *et al.*⁵ have reported the discovery of a low redshift quasar identified with the X-ray source 4U0241+61. Margon and Kwitter⁶ have reported detailed visual spectrophotometry of this quasar, 0241+622, and likewise find that its spectrum is typical of low redshift quasars. Because of the importance of establishing any morphologically unique characteristics of X-ray quasars, these quasars should be observed at all possible wavelengths. We report here IR observations of the two newly discovered X-ray quasars. These observations indicate that the X-ray quasars have IR properties typical of low redshift quasars, and, along with 3C273, are among the brightest known quasars at IR wavelengths.

All observations reported here of 4U0241+622 and MR2251-178 were obtained on the 5 m Hale telescope on Palomar Mountain. Broadband photometry from 1.25 to 20 μ m of 0241+622 was obtained on 26 March 1978 UT and again on 20 July 1978 UT. Photometry from 1.25 to 10 μ m was obtained of MR2251-178 on 17 July 1978 UT and 20 July 1978 UT. These data are plotted in Fig. 1 along with the flux observed from 3C273 (ref. 7).

Spectrophotometric observations of 0241+622 from 1.5 to 2.5 μ m with a resolution $\Delta\lambda/\lambda \sim 0.05$ using a cryogenically cooled circular variable filter wheel spectrometer were obtained on 26 March 1978 UT; these observations were repeated on 20 July 1978 UT with a circular variable filter wheel of resolution

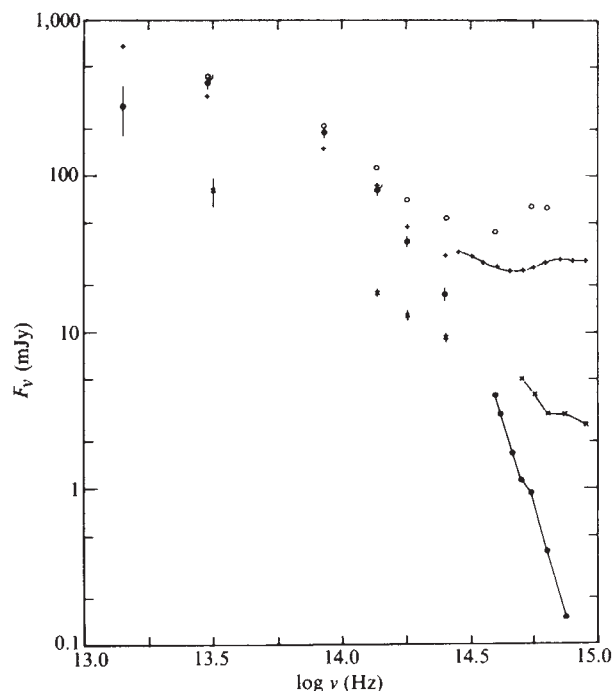


Fig. 1 The IR and visual energy distributions of the quasars 0241+622, MR2251-178, and 3C273 plotted against frequency. The data for 3C273 (+) is from ref. 7, the optical data for 0241+622 (●) is from ref. 6 and the optical data for MR2251-178 (x) is from ref. 4. ○ represent the de-reddened energy distribution of 0241+622, assuming $A_v = 4.6$ mag.

$\Delta\lambda/\lambda \sim 0.01$. Both sets of observations are shown in Fig. 2. The data were calibrated and corrected for atmospheric extinction using observations of the nearby B2Ia star 10 Persei. The data suggest that $P\alpha$ emission redshifted to $1.956 \mu\text{m}$ is present, but the atmospheric extinction limits the accuracy of the determination of the line flux of $P\alpha$.

Before the IR energy distributions of these quasars can be compared, the observed flux from 0241+622 must be corrected for significant galactic foreground extinction. Margon and Kwitter⁶ adopt a value of visual extinction $A_v \sim 4.6$ mag based on five separate estimates of the extinction. The present broadband observations give an additional estimate of the extinction. For 10 quasars with redshifts $z \leq 0.2$, Neugebauer *et al.*⁷ find that the mean V ($2.2 \mu\text{m}$) = 3.1 mag with a spread of ± 0.4 mag. The observed V ($2.2 \mu\text{m}$) colour of 0241+622 is 6.7 mag if the flux did not vary between the times of the visual and IR observations. If the intrinsic V ($2.2 \mu\text{m}$) colour of 0241+622 is 3.1 ± 0.4 mag, the quasar thus suffers 3.6 ± 0.5 mag of differential extinction between $0.5 \mu\text{m}$ and $2.2 \mu\text{m}$. If a standard Van de Hulst number 15 reddening curve^{8,9} is applied, this implies $A_v = 4.0 \pm 0.6$ mag, consistent with the value adopted by Margon and Kwitter.

A further, though weak check on the extinction is obtained from consideration of the $P\alpha$ line flux. The flux in the $P\alpha$ line based on the data in Fig. 2, is $3 \times 10^{-16} \text{ W m}^{-2}$; the determination is uncertain because of the difficulty in determining the true continuum and in assessing the line flux shortwards of $1.96 \mu\text{m}$. The $H\beta$ flux as measured by Margon and Kwitter is $\sim 4.5 \times 10^{-17} \text{ W m}^{-2}$, giving a ratio $P\alpha/H\beta \sim 7$. The observed ratio $P\alpha/H\beta$ in 3C273 is 0.3 (refs 10, 11), in agreement with theoretical calculations if the region is optically thick to Lyman series photons¹². If 0.3 is the intrinsic value of $P\alpha/H\beta$ and if the normal reddening curve applies, a visual extinction $A_v = 3.2$ mag is implied. In view of the large uncertainty in the $P\alpha$ line flux and in the intrinsic $P\alpha/H\beta$ ratio, we consider the agreement with other determinations of A_v acceptable.

The de-reddened IR energy distribution of 0241+622, based on $A_v \sim 4.6$ mag, is shown in Fig. 1. For wavelengths $\leq 10 \mu\text{m}$ the energy distributions of all three quasars are remarkably similar and in this case cannot be distinguished from other low

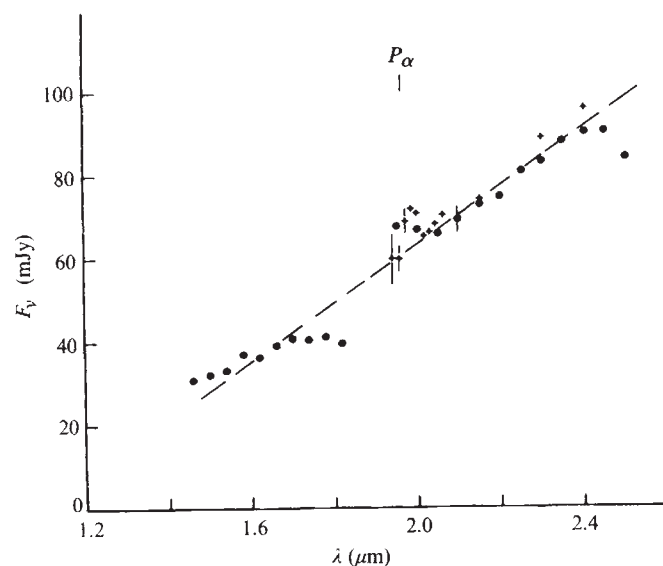


Fig. 2 The near-IR spectrum of 0241+622, plotted against wavelength. ● represent data obtained with a spectral resolution of $\Delta\lambda/\lambda \sim 0.05$, while + represent data obtained with a spectral resolution of $\Delta\lambda/\lambda \sim 0.01$.

Table 1 Emitted power per fractional bandwidth (νF_ν)

Wavelength	0241+622	2251-178	3C273
2 μm	12	4.7	120
0.5 μm	30	4.0	160
2.2 $\text{\AA}$ (5.5 keV)	2	1-9	10-50

Values are in units of 10^{37} W . The visual and IR data for 3C273 are from ref. 7, while the X-ray data for all these quasars are from ref. 5; the visual data for 0241+622 are from ref. 6; the visual data for MR2251-178 are from ref. 4.

redshift quasars. For a sample of eight low redshift quasars ($z \leq 0.2$) studied by Neugebauer *et al.*⁷, the average $2-10 \mu\text{m}$ spectral index α ($f_\nu \propto \nu^\alpha$) is -1.1 with a total spread of ± 0.3 . The intrinsic $2-10 \mu\text{m}$ index of 0241+622 is 0.9, while that of 2251-178 is 1.0. Both of these are thus well within the range of indices of the sources studied by Neugebauer *et al.*

It is of interest to compare the observed IR, visual, and X-ray luminosities of 0241+622, 2251-178, and 3C273. Table 1 shows the power per fractional bandwidth, νF_ν , where ν is the rest frequency and F_ν is the emitted power density, for the X-ray, visible and IR wavelengths; the Hubble constant has been taken as $50 \text{ km s}^{-1} \text{ mpc}^{-1}$. These observations do not show a strong correlation between the IR, visual, and X-ray luminosities of these quasars, although to within factors of three the ratios of IR, visual, and X-ray luminosities are similar for all three quasars. Note, however, that the X-ray flux for 3C273 has varied by a factor of 5 over a 2-yr period¹³ while the visible and IR fluxes have remained essentially constant (see ref. 7); this suggests that there is no direct link between the X-ray and visual/IR emission mechanisms.

The main distinguishing feature of these quasars at IR wavelengths is their brightness. The three currently known X-ray emitting quasars are comparable in visual brightness to the brightest quasars in the compilation of Burbidge, Crowne, and Smith¹⁴ and are the three brightest observed quasars at $2.2 \mu\text{m}$ (see ref. 7). The fact that the three quasars which are known to be X-ray sources are among the brightest quasars in the optical wavelengths supports the suggestion of Ward *et al.*¹⁵ and Canizares, McClintock, and Ricker⁴, that all quasars are relatively bright X-ray emitters, and sensitive X-ray surveys should provide a powerful tool for the discovery of new quasars.

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Infrared observations of the black-hole candidate V861 Sco

V861 Sco was found to be an eclipsing binary by Cousins and Lagerwey¹ and its radial velocity curve has been determined by Walker². The primary is a B01 star, and taking suitable values for its mass the radial velocity curve suggests the unidentified secondary has a mass in the range 5–12 $M_{\odot}$. Recently Polidan *et al.*^{3,4} showed that the system may be an X-ray source; it is therefore a black-hole candidate. We therefore thought that observations at other wavelengths would be of interest and we report here IR observations from 1.2 to 3.6 μm .

V861 Sco was observed from 25 July to 17 August 1978 at the Cabezon observatory, Tenerife, with the 1.5 m IR flux collector. The observations were made with the Leicester University and Royal Edinburgh Observatory IR photometers, both of which are of conventional design, use InSb detectors, and have identical interference filters. Each observation is the average of a number of 20 s integrations. The error bars for our data shown in Fig. 1 are the standard error of the mean found from these integrations. V861 Sco is very low on the southern horizon from Tenerife, and can therefore only be observed once each night.

The standard used was SAO227504 which is less than 0.5° from V861 Sco. This standard was in turn calibrated against α Cyg ($J = 1.00$, $K = 0.89$, $L = 0.78$, Johnson⁵), which was just rising at the time of observation of V861 Sco, and subsequent observations of α Cyg allows a correction for atmospheric extinction to be made. This procedure gives rise to a light curve of very reliable shape but whose absolute calibration has errors of ± 0.1 magnitudes.

The ephemeris used is calculated from the data of Cousins and Lagerwey¹ and gives the primary minimum a phase of zero.

$$\phi = (\text{JD} - 2440493.236) \times 0.127418 - 0.123$$

We have not used the ephemeris quoted by Cousins and Lagerwey¹ as this uses an epoch of JD = 2430000 which exaggerates any errors in the period. As can be seen from our data and the error in the period, this ephemeris is quite adequate at present and does not need clarifying as suggested by Polidan *et al.*⁴. Furthermore, this ephemeris places Polidan's possible X-ray eclipse at about primary minimum, when the secondary is in front of the B01a primary, which is not very satisfactory.

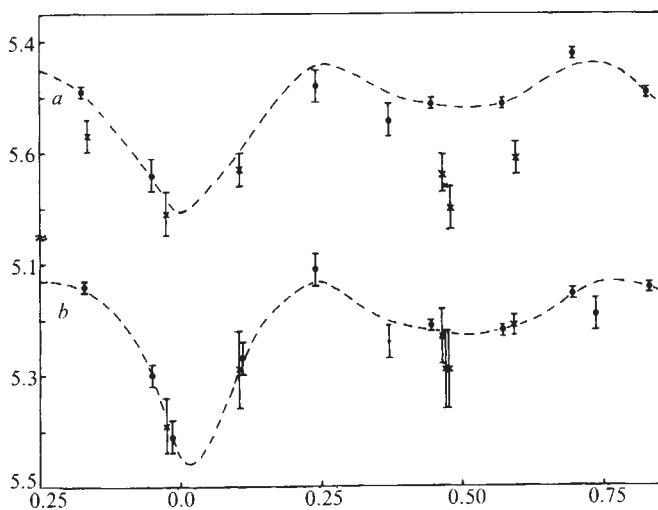


Fig. 1 Light curves of V861 Sco: a, 1.2 μm ; b, 2.2 μm . ●, Our magnitudes; ×, magnitudes from Tanzi *et al.*⁶.

Table 1 IR eclipse depths and optical data of the secondary minimum

	<i>U</i>	<i>B</i>	<i>V</i>	<i>J</i>	<i>K</i>
Δm_1	0.31	0.27	0.27	0.27	0.33
Δm_2	0.19	0.19	0.19	0.08	0.10
B_2/B_1	0.65	0.73	0.73	0.32	0.34
A_e/A_1	0.30	0.26	0.26	0.24	0.29

Figure 1 shows our 1.2 and 2.2- μm light curves and also includes the IR data of Tanzi *et al.*⁶. Their data agrees well with ours at 2.2 μm but is about 0.1 mag fainter at 1.2 μm ; this is probably due to our error in the absolute calibration due to the large air-masses. Neither our data nor that of Tanzi *et al.*⁶ is of sufficient quality to draw a useful 3.6- μm light curve.

The main interest of these observations is the shallow IR secondary minimum. Table 1 shows our best estimate of the IR eclipse depths together with the optical data taken from Cousins and Lagerwey¹. The ratio of secondary to primary surface brightness, B_2/B_1 , is calculated from these depths. We have also calculated A_e/A_1 , the eclipsed area at either primary or secondary eclipse divided by the area of the primary, assuming the eclipse is total. If the eclipse is not total this column is a lower limit for A_e/A_1 .

The rapid decrease of the surface brightness of the secondary from the optical to the IR clearly shows that this object is non-stellar. For example, if we take -0.79 for the $V-K$ colour of the B01a primary, we can show the $V-K$ colour of the secondary is -1.71 which doesn't correspond to any known star.

The non-stellar secondary is probably a compact object surrounded by an accretion disk. In the IR the cool outer regions of the disk are optically thick to free-free radiation and a low surface brightness is observed. In the visible region of the spectrum, however, the outer regions of the disk would be optically thin, allowing the hotter central region to be observed.

The presence of an optically thin accretion disk is also supported by the emission lines seen by Walker² and Polidan *et al.*³.

It may be argued that we have taken the eclipse depths at face value and not made any allowance for gravity darkening, ellipticity or heating effects on the near side of the primary. These effects can be removed by 'rectifying' the light curve and this has been done by Walker⁷ for the optical data. If the main distortion to the light curve is the ellipticity of the primary this effect should be the same in both the visible and near IR. Thus we might apply Walker's rectification to the IR light curves but, as this has an amplitude of 0.15 mag, it would more than remove the secondary minimum. Walker's rectification may therefore be excessive.

Any rectification applied to the IR light curves will only make the ratio B_2/B_1 smaller still, so our conclusion that the secondary is non-stellar remains. The shallow IR secondary eclipse also means that the area of the primary seen at phase 0.25 cannot be more than 10% greater than the area seen at phase 0.5. This small ellipticity of the primary implies that it does not fill its Roche lobe. If the primary were to fill its Roche lobe a difference of 17% would be needed; this requirement is almost independent of the assumed mass ratio. Thus the photosphere does not apparently fill its Roche lobe; however, the likely extended atmosphere of the primary could give a substantial mass transfer through the inner lagrangian point. Alternatively, if we regard the mass transfer as being due to a stellar wind then the secondary must contain a compact object, which would have to be a black hole. This conclusion is independent of any assumption about the possible X rays.

It should also be noted that if, following Walker², we take $A_v = 1.47$ the whole system has a K excess of 0.4 mag. This cannot, of course, be due to the eclipsed radiation from the secondary. If the eclipses are partial, and the secondary is effectively larger at IR wavelengths this would give the IR excess. Alternatively the whole system may be surrounded by free-free emitting material.

We conclude that these observations support the idea that the secondary of V861 Sco contains a black hole. However, a more convincing identification of the X-ray source with V861 Sco is needed. Even if V861 Sco does not turn out to be an X-ray star it would still seem to be an interesting system. Either way the IR data should assist with any future models of the system.

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HEAO 1 observations of high-energy X rays from 3C273

THE first detection of high-energy (13–120 keV) X rays from the vicinity of the quasar 3C273 is reported here. This object is one of three quasars associated with X-ray sources^{1–3}. Low energy (2–10 keV) X rays^{3–5} and γ rays (50–500 MeV)⁶ have been previously observed.

The present observations were made with the 13–180 keV slat collimated detectors of the high energy X-ray and low energy γ -ray (A4) experiment on the HEAO 1 satellite⁷. These detectors each have areas of $\sim 100 \text{ cm}^2$ and fields of view of $\sim 1^\circ \times 20^\circ$ (FWHM). The narrow slats intersect on the +Y axis and are inclined with respect to the Y-Z plane of the satellite at angles of -30° (left slat) and $+30^\circ$ (right slat).

The A4 detectors were pointed at 3C273 for $\sim 6 \text{ h}$ on 30 June, $\sim 8 \text{ h}$ on 3 July, and $\sim 10 \text{ h}$ on 5 July 1978, resulting in a positive

detection. In addition, the source was observed for $\sim 20 \text{ d}$ during December 1977 and January 1978 in the scanning mode (satellite spinning). During each rotation of the satellite the source appears a maximum of $\sim 10 \text{ s}$ in the field of view; however, there was no detection of this source.

During the pointed observations the detectors were alternately directed at 3C273 and at a reference position $\sim 4^\circ$ from the quasar to obtain background data. Background data during the scanning observations were obtained from data adjacent to source crossings. Individual photon events were pulse height analysed in 64 channels from ~ 13 to $\sim 180 \text{ keV}$ and binned in five coarse energy bands from 13–25, 25–45, 45–85, 85–120, and $>120 \text{ keV}$. Count rates in these bands were computed by averaging the background-subtracted data corrected for detector response, assuming a source at the position of 3C273 and using the Goddard Space Flight Center (GSFC) attitude solution. No data were used when the Earth or other known X-ray sources were in the field of view. A comparison of the GSFC attitude solution with the more accurate solution of the scanning modulation collimator (A3) experiment for several orbits indicated no significant ($>5 \text{ arc min}$) differences. The hard X-ray source is localised to a region of $\sim 4.5 \text{ deg}^2$ which includes 3C273.

The results of the observations are summarised in Table 1. The June/July 1978 data show statistically significant fluxes independently in each detector of $\sim 8\sigma$, $\sim 6\sigma$, $\sim 4\sigma$, and $\sim 3\sigma$ in the four energy bands from 13 to 120 keV. The December 1977/January 1978 scanning data yield only an upper limit (our detection was only at 2σ level of confidence) combining all data from ~ 13 to $\sim 120 \text{ keV}$. The ratio of the count rate in December 1977/January 1978 to the rate in June/July 1978 is 0.8 ± 0.5 . We note that the X-ray flux of 3C273 is variable on a time scale of months^{8–9} and that during our June/July 1978 observations 3C273 was in a high state⁹.

The count rate spectrum for the June/July 1978 observations is shown in Fig. 1a. Figure 1b shows the inferred photon spectrum, derived from detector efficiency curves determined by multiple scans over the Crab Nebula. Previous measurements at lower X-ray energies⁵ and at γ -ray energies⁶ are also included for comparison. The combined Ariel V and HEAO A4 data (in the region of 15 keV) indicate variability. The HEAO A4 data taken alone are consistent with a single power law photon spectrum with spectral index $\gamma = 1.67 \pm 0.14$ (1σ error). If we include the COS B data in the fit the spectral index is 2.05 ± 0.03 (1σ error).

The HEAO 1 cosmic X-ray (A2) experiment and the COS B experiment also observed 3C273 during June and July 1978 (not shown in Fig. 1). The COS B data (50–500 MeV) are consistent with their measurements in June 1976 but an intensity decrease

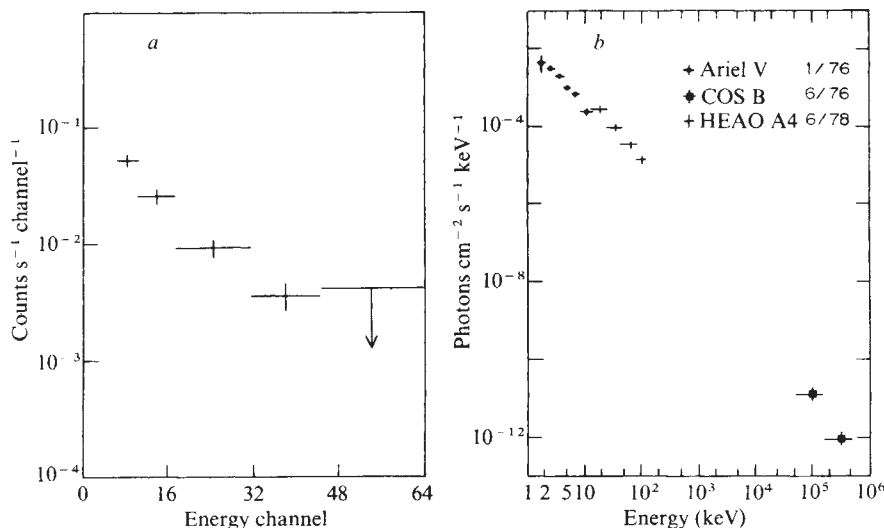


Fig. 1a HEAO A4 count rate spectrum for the June/July 78 observations. Only left slat detector data are shown. Errors are $\pm 1\sigma$ and include estimates of systematic errors due to background variations. Upper limits are at a 2σ confidence level. **b**, Photon spectrum from 1 keV to 500 MeV (see text for refs). HEAO A4 spectral points are obtained by combining left slat and right slat data. Errors are $\pm 1\sigma$ and include estimates for the uncertainty in converting from counts to photons.

Table 1 3C273 observational parameters

December 1977/January 1978

Left slat

only:	keV	
PHA count rate*	13-120	$(1.1 \pm 0.6) \times 10^{-2}$ ct s ⁻¹ per channel

June/July 1978

Left slat:

PHA count rate*	13-25	$(5.1 \pm 0.6) \times 10^{-2}$ ct s ⁻¹ per channel
	25-45	$(2.5 \pm 0.4) \times 10^{-2}$
	45-85	$(0.9 \pm 0.2) \times 10^{-2}$
	85-120	$(0.3 \pm 0.1) \times 10^{-2}$
	>120	$<0.4 \times 10^{-2}$
	13-120	$(1.4 \pm 0.2) \times 10^{-2}$

Right slat:

PHA count rate*	13-25	$(4.2 \pm 0.7) \times 10^{-2}$ ct s ⁻¹ per channel
	25-45	$(2.2 \pm 0.3) \times 10^{-2}$
	45-85	$(0.8 \pm 0.2) \times 10^{-2}$
	85-120	$(0.3 \pm 0.1) \times 10^{-2}$
	>120	$<0.3 \times 10^{-2}$
	13-120	$(1.3 \pm 0.2) \times 10^{-2}$

Photon flux†

	13-25	$(2.8 \pm 0.4) \times 10^{-4}$ photons cm ⁻² s ⁻¹ keV ⁻¹
	25-45	$(1.0 \pm 0.2) \times 10^{-4}$
	45-85	$(3.6 \pm 0.6) \times 10^{-5}$
	85-120	$(1.5 \pm 0.4) \times 10^{-5}$

*Errors ($\pm 1\sigma$) include an estimate of systematics due to background variations.

†Combined data from the right and left slat. Errors ($\pm 1\sigma$) include an estimate of systematics due to the conversion from counts to photons.

by as much as a factor of 2 cannot be excluded (Swanenburg, personal communication). The A2 data (2-60 keV) agree well with the A4 observations at 20 keV but indicate a flatter spectrum at lower energies. No single power law spectrum in the range 2-60 keV yields a satisfactory fit to the A2 data. However, if a fit to a single power law is forced, the fit yields a spectral index of ~ 1.4 (ref. 10).

A comparison of our data with those at lower X-ray energies and higher γ -ray energies indicates that there is an overall trend for spectral steepening from low to high energies with perhaps a break near ~ 20 keV. It is possible that the high energy γ -rays do not originate from the same region as the X rays¹¹. This should be considered in any comparison of spectral data in the X-ray and γ -ray regions.

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Energy sources for X-ray cluster emission

SOME galactic clusters are strong X-ray emitters¹, and we consider here the problem of establishing certain models for the source of this radiation. In the first model we suppose that the X rays are thermal bremsstrahlung from a hot quasi-isothermal inter-cluster gas. Typical parameters for the temperature, particle density and total mass of the gas are² $T \sim 3 \times 10^8$ K, $n \sim 10^{-3}$, $M \sim 3 \times 10^{44}$ kg. If we assume³ relative nuclei solar abundances, then the thermal energy contained in the gas is $E_T \sim 2 \times 10^{64}$ erg. This is $\sim 10\%$ of the total rest mass energy of a $10^{11} M_\odot$ galaxy and clearly poses a problem for potential energy sources.

To check the necessity of having a 'current' energy source (light times away) one must consider the cooling rate of the gas and compare it with its estimated age. The essential kinetic energy loss is from free-free bremsstrahlung transitions and particle-ion inelastic collision which excite ion-electron levels. However, the above kinetic energy is so high, $1.5 kT \sim 40$ keV that few ions exist with many electrons. For example, Fe XXVI, the hydrogenic iron ion, is bound by only 9.2 keV. Taken together with the small number of heavy nuclei present at all, the latter cooling mechanism is completely negligible. For a constant density plasma then, the cooling rate is⁴

$$\frac{3}{2} nK \frac{dT}{dt} = 1.42 \times 10^{-27} n_e \sum_i n_i Z_i^2 \left(\frac{T}{K} \right)^{1/2} \text{ erg cm}^{-3} \text{ s}^{-1} \quad (1)$$

where n is the total number of free particles per cm³ and n_i is the density of nuclei of charge Z_i . We consider only electron-nucleus collisions as in the non relativistic approximation ($T \ll 6 \times 10^9$ K) the radiation from electron-electron collisions is much weaker⁵. Taking $n = 4.6 \times 10^{-3}$ cm⁻³ as a typical value with $n_{\text{He}} = 0.1 n_{\text{H}}$ the temperature falls according to

$$T(t) = \{T_0^{1/2} - 157.5 t\}^2 \text{ K} \quad (2)$$

where t is in 10^9 yr and T_0 is the initial temperature in K. If we take $T_0 = 9 \times 10^8$ K then after 10^{10} yr the gas cools by only 10%. We conclude then that the hot gas may be very old, heated from its past with no current source necessary. We seek, therefore, energy sources within the cluster that were operating in the remote past and had short astronomical lifetimes with efficient energy production. The quasars are natural candidates. Due to their high intrinsic luminosities $\sim 10^{45} - 10^{48}$ erg s⁻¹, their lifetimes are necessarily short $\sim 10^8$ yr. As there are few quasars of small redshift (assumed cosmological) the quasar period of galactic evolution must be associated with young galaxies. One advantage of the quasar model is the following. If the heat input was too large, then the gas would no longer be gravitationally confined in the cluster. Instead it would envelop them. Abell clusters 399 and 401 may be examples of the overheating case⁶. Conversely, if the heat input was too small then the cluster

would not be an X-ray emitter at all. This would explain why some clusters have little or no X-ray emission. If we assume that the gas is at least as old as the galactic cluster we can estimate the age of the cluster in this model. Schmidt⁷ estimates that the space distribution of quasars is $10^{-6}(1+z)^6$ quasars Mpc^{-3} , where z is the redshift. This is valid up to $z \approx 2.5$. Assuming⁸ that the space density of galaxies is 0.03 galaxies Mpc^{-3} requires that the number of quasars relative to the number of galaxies at redshift z is $dN_{\text{rel}}/dz \approx 3 \times 10^{-5}(1+z)^6$. Thus we expect $4.3 \times 10^{-6}(1+z_0)^7$ relative number of quasars to have existed between $z = z_0$ and $z = 0$. If the energy per quasar which goes into heating the surrounding gas is R we deduce that the age of the cluster z_c in units of z is

$$z_c = (5.84) \left(\frac{E_{\text{r}}}{RS} \right)^{1/7} - 1 \quad (3)$$

where S is the number of galaxies in the cluster. Values of z greater than or less than z_c of equation (3) give a gas too hot or too cold than is observed. z_c depends weakly on the unknown number R . One may estimate this as follows. The only way that a completely ionised dilute intercluster gas can be effectively heated to such high temperatures is from collisional heating. Charged particles, cosmic rays, streaming out of quasars are the primary heating mechanisms in this model. According to Ipavich's analysis⁹ the maximum cosmic ray luminosity for a giant $10^{12} M_{\odot}$ galaxy is $10^{45} \text{ erg s}^{-1}$ for 10^8 yr yielding $3 \times 10^{62} \text{ erg}$. Taking into account that only a small percentage of the cosmic ray flux deposit their energy within the intercluster gas we estimate that a reasonable $R \sim 10^{61} \text{ erg}$.

We have applied the above analysis to Cluster Abell 2218. The parameters of this source have been given by Birkinshaw¹⁰. We take $T \sim 3 \times 10^8 \text{ K}$; $M_{\text{gas}} \sim 3 \times 10^{44} \text{ kg}$; $n \sim 4.6 \times 10^3 \text{ cm}^{-3}$; $S \sim 6.2 \times 10^4$. Then from equation (3)

$$z_c (\text{Abell 2218}) \approx 2.24 \quad (4)$$

This corresponds ($H = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$) to an age of $16.2 \times 10^9 \text{ yr}$.

The second model that we consider is a specific realisation of Katz's suggestion¹¹ that the X-ray emissions are from many distinct 'galactic type' sources rather than from an all pervading hot intergalactic gas. According to Rees¹², the 2.7° microwave background is not attributed to the cooling of big bang primordial radiation but to a later $t \sim 10^6$ – 10^7 yr epoch of intense stellar nucleosynthesis and the observed ratio $N_{\text{e}}/N_{\text{b}} \sim 10^8$ is naturally derived. An essential prediction of the Rees' cosmology is the appearance of dead remnants of massive stars strewn throughout the Universe, being the relics of the first few millennia. It seems reasonable that such objects could form large dead star clusters. In particular, one could imagine an extensive system composed of a black hole cluster with attendant matter in the form of captured gas and galaxies. This would then form the missing virial mass. A small percentage of the black holes would be in binary systems with a normal star, recently condensed out of the cluster medium. Taking $10^{37} \text{ erg s}^{-1}$ as the average X-ray luminosity of a black hole binary we would require $\sim 10^6$ – 10^8 binary systems within a cluster. If the black hole cluster is the virial mass defect then $10^{15 \pm 1} M_{\odot}$ per cluster is in the Rees' remnants: if each has an average mass of $100 M_{\odot}$ then only 10^{-8} – 10^{-4} of the available remnants need be in X-ray binary systems. Alternatively, the remnants may be supermassive and accreting matter from a diffuse uniform density ρ environment. The accretion rate is $\rho \pi R^2 v$. For rates 10^{-10} – $10^{-8} M_{\odot} \text{ yr}^{-1}$ (giving typical black hole luminosities) we require 10^4 – 10^5 black hole remnants with mass $M \sim 10^{11}$ – $10^{12} M_{\odot}$ for $\rho \sim 10^{-3} \text{ protons cm}^{-3}$. The interpretation then is that the extensive X-ray emission from galactic clusters is a physical manifestation following from the Rees' cosmology: this has a dramatic and testable prediction. Because of the rotating black hole sink, the accretion disks have a strong density gradient of optically thin to optically thick material¹³. Consequently the spectrum is expected to consist of a thermal bremsstrahlung low energy part from

the optically thick region and a high energy inverse Compton part from the optically thin region. The spectrum therefore should drop with a black body slope to $\approx 50 \text{ keV}$ and then begin to increase to a broad (comptonised) hump out to a few hundred keV. It would be desirable to have good X-ray data between the region 10 – 500 keV if X-ray cluster emission has this two component form.

Finally the two models discussed here may be unified. If the mysterious quasar source has its origin in gravitational collapse¹⁴ then a smaller black hole cluster may be the remnant of early quasar activity.

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Auto-irradiation effects in DT pellets

FUSION reactors based on magnetic containment and operating in a quasi steady-state will need to be refuelled during their burn period. The ballistic injection of solid pellets of hydrogen isotopes, first suggested in 1954¹, still seems to be the most promising method, and two satisfactory techniques for fabricating solid hydrogen pellets have been developed. In one, a continuous extrusion of solid hydrogen is thermally chopped into cylindrical pellets². In the other, a jet of liquid hydrogen is acoustically broken into uniform droplets which are then frozen by reducing the pressure to below the triple point value³. Both techniques have worked well with deuterium, but fusion reactors will need DT pellets, and we consider here how the radioactivity of tritium will affect the situation. Two effects are apparent with characteristic times which may be short enough to be of practical significance: thermal stress arising from the deposition of decay heat throughout the pellet and electric stress caused by the charging of the pellet due to the escape of β particles from its surface. For a pellet of radius a , the thermal stress varies as a^2 and the electric stress as a^{-2} , which leads to both upper and lower size limits at radii where the effective stress approaches the yield stress of solid hydrogen. Fortunately the range of pellet radii envisaged in current reactor concepts (10^{-2} – 10^{-4} m) falls mid-way between the two limits.

The heat produced by the β decay of tritium in an equimolar DT mixture is 1.0 W mol^{-1} . In a thermally insulated sample this would cause a uniform temperature rise, but in pellets prepared by the techniques mentioned and injected into a cold vacuum chamber, there will be radial temperature gradients caused by

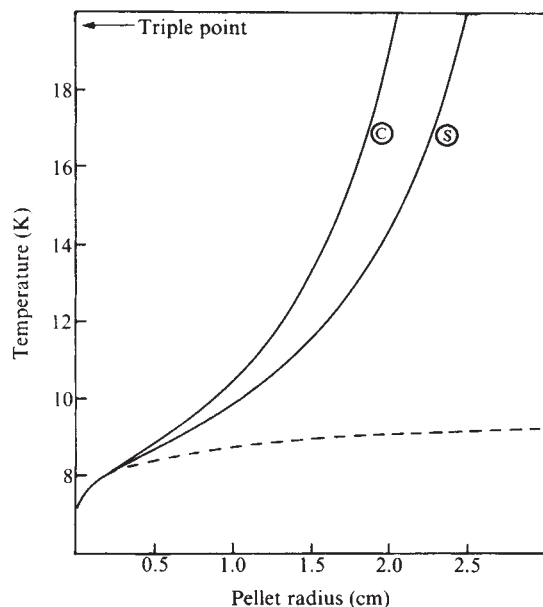


Fig. 1 Variation of equilibrium temperatures with pellet size. The solid curves are the central temperatures for cylindrical © and spherical © pellets. The dashed curve is the surface temperature. The triple point temperature of DT is 19.8 K.

outward heat flow. This can happen during extrusion by conduction through the nozzle; in isolated pellets evaporation establishes an equilibrium surface temperature which increases with pellet radius. By equating evaporative loss with decay heat we find this to be 7.0–8.7 K for the size range quoted above; radiative heat transfer is negligible in these conditions. To arrive at the equilibrium radial temperature distribution we assume the pellets to have constant surface temperature T_a and uniform heat generation A_0 , and a thermal conductivity K which varies with temperature. This leads to a parabolic distribution of the form:

$$\int_{T_a}^{T_r} K dT = \frac{A_0}{C}(a^2 - r^2)$$

where the numerical factor C is 4 for a cylinder and 6 for a sphere⁴.

The thermal conductivity of solid DT is unknown, but it has been suggested that when freshly frozen it may behave like 72% *para* H_2 (ref. 5). The variation of central temperature with pellet size shown in Fig. 1 is calculated on this assumption, and suggests that pellets smaller than about 2 cm radius are unlikely to have molten centres.

The thermal stress at the surface of a pellet whose temperature is a function only of radius is proportional to the mean temperature minus the surface temperature⁶, that is

$$(\sigma)_a = \frac{\alpha E}{(1-\nu)} \{\bar{T} - T_a\}$$

where α is the linear expansion coefficient, E is Young's modulus and ν is Poisson's ratio. By averaging the parabolic temperature distribution we find the equilibrium surface stress to be:

$$(\sigma)_a = \frac{\alpha E}{(1-\nu)} \cdot \frac{A_0 a^2}{DK}$$

where the numerical factor D is 8 for a cylinder and 15 for a sphere. The growth rate of this stress is a function of the dimensionless quantity $\kappa t/a^2$, where κ is the thermal diffusivity; the time t to reach 75% of the equilibrium stress is $0.25a^2/\kappa$ for a cylinder and $0.16a^2/\kappa$ for a sphere⁴.

There are no published experimental data on the mechanical or thermal properties of DT, so to estimate the surface stress and

Table 1 Data for thermal stress calculation

Quantity	Unit	$T = 5$ K	$T = 10$ K	Material	Ref.
B_s	$\text{mol m}^{-3} \text{K}^{-3}$	0.26	0.26	DT	5
H	W mol^{-1}	1.0	1.0	DT	
αA_0	$\text{W m}^{-3} \text{K}^{-1}$	6.6	26	DT	
K	$\text{W m}^{-1} \text{K}^{-1}$	0.2	0.8	nH_2	5
$(1-\nu)$		0.7	0.7	nD_2	7
E	MN m^{-2}	460	420	D_2	8
κ	$\text{cm}^2 \text{s}^{-1}$	0.14	0.083	nD_2	9
Stress					
cylinder	GN m^{-2}	$2.7a^2$	$2.4a^2$		
sphere	GN m^{-2}	$1.4a^2$	$1.3a^2$		
(t)_{75%}					
cylinder	10^4 s	$1.8a^2$	$3.0a^2$		
sphere	10^4 s	$1.2a^2$	$1.9a^2$		

To evaluate the stress we note that the solid density of hydrogen closely obeys the empirical relationship⁵ $(\rho_s)_T = (\rho_s)_0 - B_s T^3 \text{ mol m}^{-3}$, whence $\alpha = B_s T^2 / \rho_s$. As $A_0 = H \rho_s$, we have $\alpha A_0 = H B_s T^2$.

its growth rate we have used a judicious mixture of measured data for other solid combinations of the hydrogen isotopes together with some predictions for DT based on them. The values used to plot the graphs in Fig. 2 are given with their sources in Table 1.

Electrical stress on isolated pellets arises from charging due to the escape of β particles born sufficiently near the surface and having outwardly directed velocities. The electrostatic energy of a sphere with a surface charge Q is $\mathcal{E} = Q^2/8\pi\epsilon_0 a$ and hence the outward electrostatic pressure at the surface is

$$P = -\frac{d\mathcal{E}}{dv} = Q^2/32\pi^2\epsilon_0 a^4$$

If its radius a is small enough, a pellet will charge up to a value Q_{crit} beyond which disruption occurs; we suggest that the critical pressure at this point can be identified with the yield stress. It is

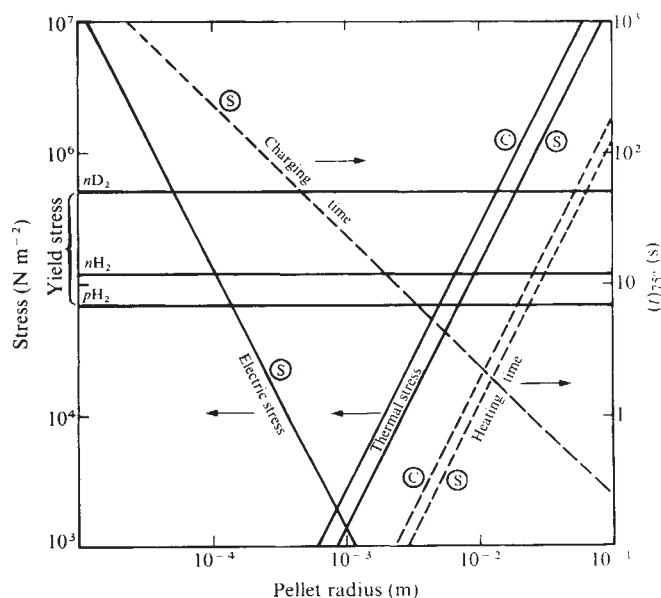


Fig. 2 Stress in DT pellets. The solid sloping lines show the steady state electrical and thermal stresses. The broken lines show the times to reach 75% of these stresses. © refers to cylindrical pellets, © to spherical. The pellet surface temperature is 5 K. The horizontal lines are measured yield stresses for polycrystalline hydrogen.

analogous to the Rayleigh stability criterion for charged liquid droplets¹⁰, which can be written:

$$\gamma \geq Q^2/64\pi^2\epsilon_0 a^3$$

where γ is the surface tension. If pellets behave like droplets, we may expect further charging to expel from the surface small fragments carrying sufficient charge to restore the stability criterion $Q \leq Q_{\text{crit}}$ ¹¹. For sufficiently large radii Q_{crit} will not be reached, as it would imply a potential greater than that corresponding to the maximum β energy ($V_{\text{max}} = 18$ keV); such pellets will be electrostatically stable.

The rate of charging is $dQ/dt = e(dn/dt)$, where dn/dt is the electron loss rate from the surface. It has the initial value:

$$\left(\frac{dn}{dt}\right)_0 = 4\pi a^2 RS$$

where R is the thickness of a hypothetical layer from which all β particles are thought to escape and S is the activity. As the potential of pellets of all radii rises towards V_{max} asymptotically, it is convenient to make quantitative assessments in terms of their potential V . Using the relationship $Q = 4\pi\epsilon_0 a V$, we have:

$$\left(\frac{dV}{dt}\right)_0 = eRS/\epsilon_0$$

which we evaluate by taking R to be approximately the half-range for self absorption in tritium, quoted by Evans¹² as 0.039 mg cm^{-2} ; the value of S for an equimolar DT mixture is $2.13 \times 10^{17} \text{ decays kg}^{-1} \text{ s}^{-1}$. This gives:

$$\left(\frac{dV}{dt}\right)_0 = 1.5 \times 10^6 \text{ V s}^{-1}$$

The time taken to charge to a given fraction of V_{max} will depend on the β energy spectrum, but we consider it sufficiently accurate for these estimates to assume it to be flat, so that:

$$V = V_{\text{max}} \left\{ 1 - \exp \left[- \left(\frac{dV}{dt} \right)_0 t / V_{\text{max}} \right] \right\}$$

Figure 2 shows the equilibrium electric stress,

$$P_{\text{max}} = \epsilon_0 V_{\text{max}}^2 / 2a^2 = 1.4 \times 10^{-3} / a^2 \text{ N m}^{-2}$$

and the charging time to 75% of P_{max} ,

$$(t)_{75\%} = 2.4 \times 10^{-2} / a \text{ s}$$

to facilitate direct comparison with the thermal stress.

In assessing our results the first point is that the total stress is a minimum for pellets of ~ 1 mm radius. This is a convenient size for fusion reactor concepts¹³; a total injection rate of 100 pellets per second would give a gross output of about 4,000 MW(th) at 10% burn-up per pass.

In the absence of data on the strength of solid DT, the measured yield stresses for polycrystalline H_2 and D_2 ⁸ are shown in Fig. 2. Using these figures as a guide, we see that pellets smaller than 10^{-2} m radius are unlikely to suffer early disruption due to thermal stress. Larger pellets may need to be fabricated and used within a few seconds, otherwise they may be prone to surface cracking and central melting, both of which could increase their ablation rate on entering a hot plasma. Electric stress seems to be of little consequence unless pellets are smaller than 10^{-4} m radius, where the slow charging rate will diminish its practical importance.

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Mercury(II) sulphide: a photo-stable semiconductor

THERE is much interest in photo-electrochemical cells which use a semiconductor-electrolyte junction to transduce light into electrical and chemical energy¹. The semiconductors investigated to date fall into two main groups, the first of which contains photostable material but which shows response in the UV rather than the visible region of the spectrum, for example, n-titanium dioxide², n-strontium titanate³, n-potassium tantalate⁴, n-tin oxide⁵ and n-barium titanate⁶. The second group comprises compounds which respond to the visible region of the spectrum but are photochemically unstable such as n-gallium phosphide⁷, n-indium phosphide⁸, n-cadmium sulphide⁹, and n-cadmium selenide¹⁰. Attempts have been made to extend the spectral response of the materials belonging to the first group, by doping¹¹ and chemically affixing dyes to the surface of the semiconductor¹². Some success has been attained in stabilising some of the members of the second group; for example, cadmium sulphide and selenide are stable in polychalcogenide solutions¹³. If cells containing a semiconductor-electrolyte junction are to be of any practical use in the harnessing of solar energy then the following criteria for the semiconductor should be met: (1) they should absorb visible radiation; and (2) be photostable and capable of use in powder form. Clearly, if the conduction and valence bands of the semiconductor have energies that facilitate proton reduction and oxidation of hydroxyl ions respectively this would be an added bonus as the cells could be used to either photo-assist or photo-electrolyse the water¹⁴. We report here on mercury(II) sulphide (cinnabar) band gap = 2.1 eV¹⁵ which fulfils most of the above criteria¹⁶.

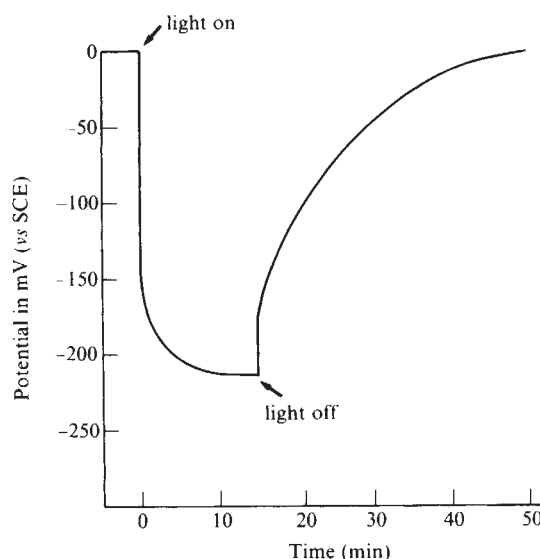


Fig. 1 Plot of potential versus time for red HgS in pH 7 (unbuffered) 0.1 M NaNO_3 , with respect to an SCE.

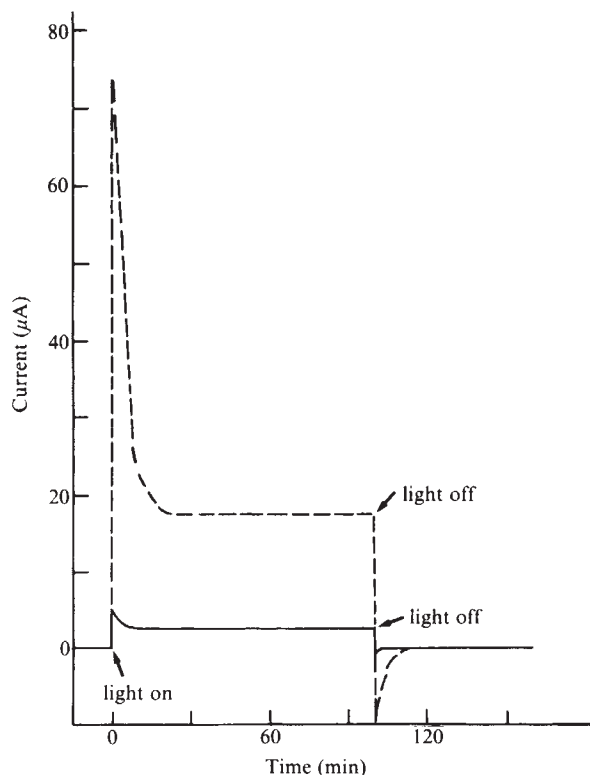
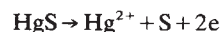


Fig. 2 Plot of current versus time for HgS (held at 0 V versus SCE) in pH 7 (unbuffered) 0.1 M NaNO₃. Solid line, untreated red HgS. Dashed line, red HgS, blackened by irradiation in potassium iodide. Effective area of electrode irradiated is 2.25 cm² and light intensity is $\sim 2.5 \times 10^{-2}$ W cm⁻².

The electrodes consist of a platinum mesh coated with cinnabar (optoacoustic spectra show that the samples absorb little radiation above 620 nm and presumably therefore, contain very little *meta*-cinnabar). The coating is carried out by dipping the mesh into a suspension of cinnabar produced by boiling a deoxygenated aqueous solution of the powder followed treatment with an ultra-sonic probe. The mesh is removed from the suspension and then dried by hot air. To obtain reproducible results the electrode is stored in an oxygen-free unbuffered 0.1 M sodium nitrate solution (pH 7) for at least 15 h. When such an electrode is placed in an electrolyte solution such as an unbuffered 0.1 M sodium nitrate solution (pH 7) and is connected to a voltmeter (impedance 10^7 – 10^8 Ω) with an SCE as reference, illumination of the electrode generates a photo-e.m.f. The light source is a 1.8 kW xenon lamp and the light is filtered by passing it through a 0.28 M copper(II) chloride solution maintained at 5 °C. This filter solution has maximal transparency at 515 nm and has a half-band width of 90 nm. The direction of the e.m.f. and plot of the photo e.m.f. versus time (Fig. 1) are all typical of the behaviour of an n-type semiconductor. The photo-e.m.f. is somewhat dependent on the history of the sample but usually falls in the range of –150 to –300 mV. When the cinnabar electrode is connected to a platinum counter electrode and a potential of 0 V maintained between the two by means of a potentiostat and a SCE reference, illumination causes a current to flow (Fig. 2). As expected, the photocurrent increases as the anodic bias is increased. From a determination of the biasing voltage at which zero current flows, the flat band potential of these electrodes was determined as ~ -0.25 V versus SCE at pH 7. This value varies with pH according to the Nernst equation (that is, ~ -60 mV per pH unit). When inert electrolytes such as sodium nitrate, are used, irradiation of such electrodes or suspension of mercury(II) sulphide does not lead to any solubilisation of mercury (as detected by conventional atomic absorption spectroscopy where the lowest reliable

detection of mercury is $7.5 \mu\text{g ml}^{-1}$). We conclude therefore, that the photocurrents are photoelectrolytic currents.

Anodic oxidation could occur on illumination:



One mercury atom should be solubilised per two electrons passed anodically. Stability of HgS was confirmed by passage of enough positive photocurrent to yield a theoretical mercury concentration of $62 \mu\text{g ml}^{-1}$ in the electrolyte: atomic absorption analysis showed that within the detection limit, no mercury solubilisation by anodic oxidation occurs.

Solubilisation of mercury does, however, occur when the electrolyte contains easily oxidisable ions, such as iodide. With these ions, high currents are produced and the red cinnabar changes colour and becomes brownish-black. Preliminary studies have shown that mercury(II) sulphide undergoes a photochemical reaction with iodide and other reducing ions, but as yet the products have not been identified. However, when the darkened electrodes are removed from the electrolyte, washed well with distilled water and then used in an inert electrolyte, no further solubilisation of mercury occurs. Blackening of electrodes by this procedure enables one to prepare electrodes which give significantly higher currents at zero bias ($\sim +15 \mu\text{A}$) than the untreated cinnabar electrodes (see Fig. 2). A bonus is that the darkening extends the spectral response. The nature of the material responsible for the dark colour is uncertain, although X-ray powder photographs and optoacoustic spectra suggest that some of the colour is due to presence of *meta*-cinnabar.

Electrodes which deliver high currents at zero bias are also effective in photo-assisting the electrolysis of water. Thus at pH 12, using degassed sodium nitrate or chloride as electrolyte and a bias of $> +0.2$ V (versus SCE) leads to gas evolution at the platinum counter electrode and gas bubbles on the semiconductor surface can often also be seen.

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An auroral effect on the fair weather electric field

EVIDENCE is presented here for coupling between the upper and lower atmosphere by electrical means related to aurorae. Electric field data obtained with rocket-launched parachute-borne probes are shown to be consistent with the hypothesis that aurorally-produced radiation can short out vertical electric field 'cells' in the mesosphere, with corresponding effects on the lower atmosphere electric field.

Effects of solar sunspot-related activity on the lower atmosphere electric field occur too frequently to be explained by direct modulation of the 'classical' global electrical circuit¹, which requires large variations in galactic cosmic-ray flux or rare solar cosmic-ray events which penetrate to the lower stratosphere². Available data indicate responses of the lower atmosphere field to much smaller and more frequent solar and geomagnetic disturbances². Israël¹ reported observations of the direct effects of aurorae on the surface field by early investigators, and more recent observations of such effects, including balloon measurements, are cited in refs 2 and 3.

One mechanism which may explain some such observations involves the existence in the mesosphere of a large vertical electric field, with a total potential of the order of 100 kV.

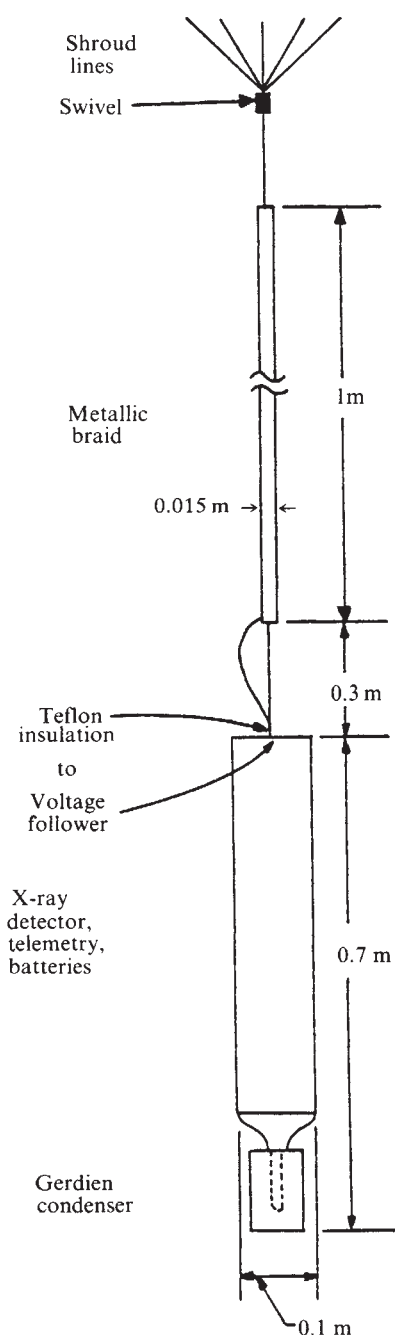


Fig. 1 Electric field antenna configuration.

Where this mesospheric field is shorted by ionisation produced by radiation, then this potential may be added to the lower atmosphere potential (~ 300 kV), producing an increase or decrease depending on the direction of the mesospheric field. Large mesospheric fields were first observed in the USSR⁴. Sceptical of this result, we concluded that such fields should have appeared as base-line shifts in previously obtained conductivity measurements using parachute-borne electrical conductivity probes. On checking old data we found evidence of such base-line shifts in several cases⁵, with the largest electric field effect

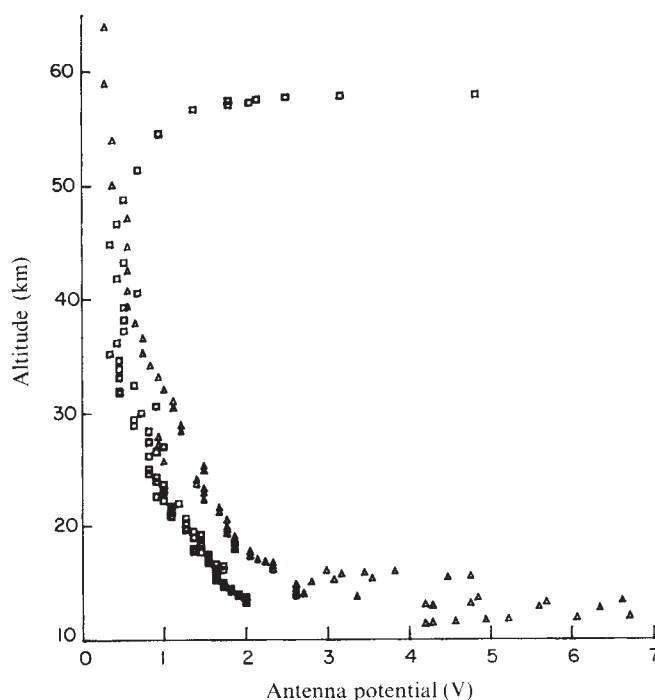


Fig. 2 The variation of antenna potential with altitude: □, quiet, 22 March 1978 06.52 UT; △, disturbed, 27 March 1978 10.26 UT.

appearing in a measurement made in Chilca, Peru, near the magnetic equator. (This may indicate that the fields are generated primarily at low latitudes.) In operation Aurorazone I, aimed at measuring the atmospheric effects of aurorae at Poker Flat Research Range near Fairbanks, Alaska, in September 1976, it was observed that a baseline shift indicating a large electric field was present only during quiet conditions, vanishing during disturbed periods of auroral activity (J. D. Mitchell, personal communication), presumably due to enhanced electrical conductivity. This led to the hypothesis that observed lower atmosphere effects were due to the shorting out of a permanent electrical field cell in the mesosphere, with the associated potential appearing in the lower atmosphere circuit⁶. The existence of such large mesospheric fields may be related to aerosol layers, which make the atmospheric ionisation relatively immobile⁷.

To investigate this phenomenon further, electric field measurements were conducted at Poker Flat during operation Aurorazone II in March 1978. The instruments used 1-m metallic antennas above parachute-suspended payloads, as shown in Fig. 1. Teflon insulation was used to connect the antenna to an electrometer follower (Intersil ICH 8500A). The payload section included telemetry, and also X-ray detectors and a Gerden condenser for charged particle measurements (provided by other experimenters). This inherently asymmetric system is capable of measuring relative electric fields at night, when photo-emission from surfaces is unimportant.

Figure 2 shows data obtained from two such launches on Super Arcas rockets. The data, in potential difference between

the antenna and payload (accurate to ± 0.3 V), are approximately the same as the downwards electric field in V m^{-1} , the effective length of the dipole being of the order of 1 m. The data show the field during a very quiet period (22 March, 06.25 UT) and during a disturbed condition (27 March, 10.26 UT) which occurred following the onset of a geomagnetically disturbed period when the Poker Flat riometer indicated over 5 dB of enhanced galactic noise absorption, with extensive visible aurora. There was no reported thunderstorm activity or precipitation (rain or snow) in Alaska during these periods (H. Cole, personal communication).

The quiet data indicate the normal fair weather pattern at lower altitudes, but show an enhancement at high altitudes. The rocket performed less well than expected, so that the peak implied by the highest altitude data points was not attained. The disturbed data indicate a small field at high altitudes, but a substantially higher field at lower altitudes, with some evidence of ragged structure. If the ionosphere is nearly equipotential at a higher altitude, say 100 km, then shorting the mesospheric part of the circuit would be expected to produce a correspondingly altered field in the lower atmosphere, as is observed.

This effect could penetrate to a lower latitude as the amount of radiation necessary to short the mesospheric field may be relatively small. Beyond this the effect would decrease towards lower latitudes as determined by mapping of the field. A more complete global description of the electrical response of the atmosphere to geomagnetic disturbances would require that future auroral zone 'campaigns' be supplemented by measurements at various latitudes. Because of the inferred importance of mesospheric fields to the complete global circuit this would require rocket measurements supplemented by balloon and surface measurements. The most reasonable way of acquiring mesospheric data would seem to be using low cost meteorological rockets as are widely used for wind and temperature measurements, utilising electric field and charged particle sensors deployed on parachutes.

Note that the coupling mechanism suggested above is not necessarily the only one of importance. Direct modulation of the lower atmosphere field has been demonstrated in connection with large solar cosmic ray events⁸. Magnetospheric and ionospheric fields have been shown to map to low altitudes⁹. Rapid responses of the surface field to auroral events^{3,10} may be induced by aurorally produced charge separation.

We thank J. R. Barcus, R. A. Goldberg, J. D. Mitchell and J. J. Olivero for useful discussions. Assistance with rocket payloads and launch operations was provided by personnel of NASA Wallops Flight Center and Poker Flat Research Range of the University of Alaska. D. F. Young assisted with calculations. This work was supported by NASA grant NSG-6004.

Note added in proof: Combining Gerdien condenser conductivity data (J. D. Mitchell, personal communication) with the electric field measurements shows that vertical current density increases rapidly above 30 km in both the quiet and disturbed cases. This seems to indicate that currents with density much higher than the 'fair weather' current circulate in the upper stratosphere and mesosphere, which may explain the nature of the effect described above.

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Geomagnetic activity and the high latitude magnetic field of the Sun

SMITH *et al.*¹ using Pioneer 11 in 1976 did not observe any sector structure at the heliographic latitude of about $+16^\circ$. At this latitude the field polarity was consistently directed away from the Sun. Its direction was in accord with the direction of the high-latitude magnetic field near the north pole of the Sun. This result, as well as indirect data²⁻⁴, shows that the high latitude solar field extends far into interplanetary space. The evidence for this is shown by the analysis of the yearly *aa* indices^{5,6} between 1868 and 1976. We find intensification of geomagnetic disturbances during those 11 yr periods when the extended solar magnetic field polarity in the northern hemisphere is away from the Sun.

From measurements by the Mount Wilson^{7,8}, Cambridge⁹ and Crimea¹⁰ magnetographs one finds that the general magnetic field of the Sun varies with a period of ~ 22 yr. Then assuming the first year of the 11-yr cycle and the even numbered years as the beginning of each oscillation and taking the 22-yr Hall cycle period as the unit, one finds that the high latitude field of the Sun is maximum near the phase 0.5. During this interval the field vector is in the same direction as the vector of the angular velocity of the solar rotation, that is, the magnetic field polarity is away from the photosphere in the northern hemisphere. During the interval near phase 1.0, the absolute field value attains a maximum again but its polarity near the poles reverses. The high latitude field approaches zero and reverses near the 0.25 and 0.75 phases, in the neighbourhood of the 11-year cycle maxima.

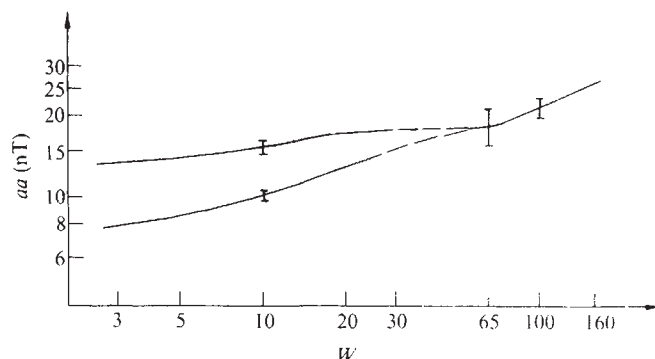


Fig. 1 The relationship of the yearly *aa* indices of geomagnetic activity with Wolf number (*W*). The lines for $W < 27$ are the approximations from the text, for $W > 60$ the *aa* values are calculated for intervals of *W*. The errors bars are shown.

The Earth migrates at 7°S – 7°N heliographic latitude throughout the year. So the Earth crosses the current sheet, which is almost parallel to the solar equatorial plane and separates the two hemispheres of opposed field polarity. Taking into account Earth's migration and the time variation of the inclination of the geomagnetic dipole, as derived in ref. 11, we find that the projection B_θ of the interplanetary magnetic field (IMF) in the direction of the geomagnetic dipole is large enough to have some geomagnetic effect. Near phase 0.5 this component B_θ is parallel to the geomagnetic dipole (antiparallel to the geomagnetic field at the magnetopause) and one may expect a reconnection¹² of the IMF and geomagnetic field. This phenomenon increases near phase 0.5 compared with phase 1.0. This must be followed by intensification of geomagnetic activity near the ends of the even cycles in comparison with ends of the odd cycles.

The variation of geomagnetic activity near solar cycle minima has been described elsewhere¹³. The geomagnetic activity index¹⁴ M used for the interval 1884–1967 was based on a regression of M with the Curie indices, and is not homogeneous throughout the whole hundred year interval. Therefore quantitatively comparing the mean M values in periods with different levels of solar activity is not reliable. The difference in mean Curie values in even and odd solar cycles has also been noted by Russell¹⁵.

The aa index introduced by Mayaud^{5,6} is a better index for long-term studies. Mayaud defined the aa index as the average of 3-h K indices converted to the amplitude of the field at two antipodal observatories. The Greenwich and Melbourne Observatories are roughly antipodal and have been gathering data since 1868. Comparing the aa indices with the planetary a_m indices shows¹⁶ that the correlation coefficient between the mean monthly and the mean annual values attains 0.994 and 0.998, respectively. Thus the aa index obtained from the magnetograms of two antipodal observatories gives a good picture of the mean monthly planetary geomagnetic activity.

For years of low solar activity ($W < 27$), the comparison of mean annual aa indices with Zürich Wolf number (W) for 1868–1975 (refs 17, 18) yielded the following linear approximations:

$$aa = (0.31 \pm 0.08)W + (6.98 \pm 1.12) \text{ nT}$$

$$aa = (0.25 \pm 0.11)W + (12.7 \pm 1.11) \text{ nT}$$

for the ends of the 11-yr cycles with odd and even numbers, respectively. The correlation coefficients between aa and W are 0.88 ± 0.06 and 0.42 ± 0.25 for odd and even minima respectively. So, the geomagnetic activity can be seen to be higher near phase 0.5 than near phase 1.0. When the Wolf Numbers vanishes ($W \rightarrow 0$) the aa index for the even cycles remains almost double that for the odd cycles. The dependence of aa on W for the sunspot minimum, together with the aa index averaged over intervals of W at sunspot maxima are shown in Fig. 1.

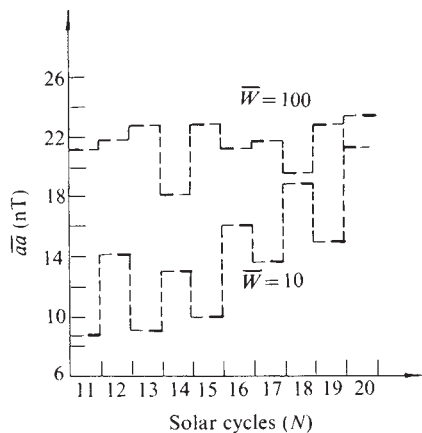


Fig. 2 The variations of the $\overline{aa}$ indices for the maxima ($\overline{W} = 100$) and the ends of the solar 11-yr cycles ($\overline{W} = 10$).

For the years near the extrema of 11-yr cycles the averaged $\overline{aa}$ and $\overline{W}$ value are used. For maxima we used the data of the three years interval, the year of maximum W and two neighbouring years, in the cycles nos 15 and 16 the fourth year with a large W was also added. For the minima data with average annual values of $W < 27$ and $aa < 20$ were used. The year 1976 is not suitable for this criterion but as it was the minimum of the solar cycle it was taken as being characteristic of the unusual cycle no. 20. For the end of cycle no. 15 we did not use the 1921 and 1922 data as

W (for 1921) and aa (for 1922) were close to the chosen limiting line. The insertion of these years gives $\overline{aa} = 14.0$ and $\overline{W} = 15.7$ for the 1921–1924 interval. The reduction to $\overline{W} = 10$ gives $aa = 12.2$, but that does not change the relationship between geomagnetic disturbance and even polarity of a cycle as shown in Fig. 2.

Table 1 Variation of Wolf numbers with annual aa indices

No. of solar cycle (N)	Maxima			Minima		
	Years	$\overline{aa}$	$\overline{W}$	Years	$\overline{aa}$	$\overline{W}$
11	1870–1872	22.5	117.3	1875–1879	8.8	10.0
12	1882–1884	18.3	62.3	1887–1890	13.8	8.3
13	1892–1894	20.7	78.6	1897–1903	10.6	15.2
14	1905–1907	14.6	59.8	1910–1914	12.4	7.8
15	1916–1919	20.6	76.3	1923–1924	10.2	11.2
16	1926–1929	18.4	68.9	1931–1934	16.4	11.7
17	1937–1939	21.9	104.3	1944–1945	17.0	21.3
18	1947–1949	23.0	141.0	1954	17.3	4.4
19	1957–1959	29.4	177.9	1964–1965	15.6	12.6
20	1968–1970	21.6	105.4	1976	23.8	12.6

The $\overline{aa}$ and $\overline{W}$ values for the cycles from no. 11 to no. 20 are given in Table 1. This shows at sunspot minima, despite the differences in $\overline{W}$, the $\overline{aa}$ indices are greater at the ends of the even cycles than at the ends of the neighbouring odd cycles. At sunspot maxima one can see only the 90-yr variations of $\overline{W}$ with essentially no differences between even and odd cycles.

Using the relationships between aa and W (Fig. 1) the $\overline{aa}$ indices were reduced to values for $\overline{W} = 10$ and $\overline{W} = 100$, at the minima and maxima of solar cycles respectively. These reduced $\overline{aa}$ values are shown in Fig. 2. The large differences in $\overline{aa}$ values seen at sunspot minima in passing from odd to even cycles are absent for sunspot maxima. The difference between the geomagnetic activity during the even and odd minima for cycles 11–20 is given by the ratio $\overline{aa}_{2k}/\overline{aa}_{2k-1} = 1.53 \pm 0.05$ (where $N = 2k$ and $2k - 1 = \text{No. of the cycle}$).

The 11-yr and 22-yr mean values of the aa index are given by Russell¹⁹ for the period 1872–1967. However, he used mean values of the cycles, and did not normalise for the difference in the levels of solar activity as measured by sunspot number. Therefore an enhancement of the aa index at the ends of even cycles as compared with the odd cycles was not confirmed.

Our investigation shows evidence of the existence of an extended solar magnetic field and its variation within a 22-yr period. The physics of this phenomenon is as follows. The temperature (and the density) of the solar corona is lower at high latitude near the sunspot minima. The weakened solar wind in this region does not affect the magnetic field. Thus the magnetic field in the vicinity of the Sun must be nearly a dipole, particularly at high latitudes. This is consistent with the structure of a sunspot minimum solar corona. Near the solar equatorial plane the kinetic and thermal energies become more than the energy of dipole magnetic field, that is $\beta = (1/2\rho U^2 + 3N_e kT)/B^2/8\pi \approx 1$ at a distance of $9R_\odot$. Consequently, the solar wind attracts the field lines near the solar equator plane at a distance of $\sim 9R_\odot$. Thus the field has one polarity in the hemisphere north of the solar equatorial plane and the opposite polarity in the hemisphere south of the equatorial plane. Assuming that the total flux for the entire polar region reaches 3×10^{21} Mx and that the magnetic field B is proportional to r^{-3} for $r < 9R_\odot$ and B is proportional to r^{-2} for $r > 9R_\odot$, we obtain $B \sim 1$ nT near the Earth's orbit.

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A radiocarbon date from Lake Bogoria, Kenya Rift Valley

RADIOMETRIC dates from faunal remains deposited in lacustrine and fluvio-lacustrine sediments at numerous locations in and around the lakes of the Kenya Rift Valley confirm a period of extended lake development related to a wetter climatic phase ~10,000 yr ago^{1–7}. Thereafter, a drier climatic regime has prevailed and the lakes have contracted, although fluctuating in rapid response to sequences of wetter or drier years. Lake Bogoria (Hannington) is the last of the Kenya Rift Valley lakes to yield a radiocarbon date for this early Holocene high stand. Although a general synchronism of maximal lake development has emerged, as we report here, there is considerable variation in the dates obtained from each lake basin (Fig. 1).

Saline-alkaline Lake Bogoria lies in a fault-controlled trough just north of the Equator^{8–9}, extends north-south for 17 km, varies in width from 500 to 3,500 m (Fig. 2), and has a maximum depth of ~9 m¹⁰. It is sharply delimited by alkaline lavas of Miocene to Pleistocene age^{8–9}, except on its northern margin, where the 1–10 km wide, swamp-covered, Lobo Plain leads for 20 km north across a low watershed to fresh water Lake Baringo. Short ephemeral streams and the semi-permanent Sandai River drain into Lake Bogoria, which also receives water from three groups of hot springs and small geysers. Lake Bogoria has no proven outlet, although underground seepage to the north is likely⁸. Fluvio-lacustrine sediments fringe the lake margins, but are seldom more than a few tens of metres wide except at the northern end. A grey, stromatolitic limestone that coats *in situ* bedrock and clastic sediments up to ~11 m above present lake level, and fossiliferous silts, are the principal evidence for a formerly more extended lake. Excepting algae, no organic life is known to exist in Lake Bogoria today, although crocodiles and hippopotami were photographed swimming in

the lake in 1927 (ref. 11). Mollusc shells, crocodile, hippopotamus and fish bones, and diatoms in fluvio-lacustrine sediments up to 5 m above present lake level, indicate a formerly richer fauna/flora and suggest a fresher water environment. Today the pH of Lake Bogoria exceeds 10.8 and the Na⁺ concentration reaches 28,400 p.p.m.^{8,12}.

A 250 g sample of *Melanoides tuberculata* was collected from the lower part of a 2 m thick fluvio-lacustrine unit of feldspathic silts with pebble bands, the base of which varies from 2.95 to 3.10 m above the July–August 1976 level of Lake Bogoria (Fig. 2). The state of preservation of the shells was good and they were believed to be *in situ* rather than derived. After dissolving and discarding the outer layers of the shells (~20%), dates of 10,560 ± 170 yr BP and 10,320 ± 150 yr BP (Birm-883) were obtained for the inner and middle fractions respectively. In each case the final figure is a conventional radiocarbon date, calculated using a half life of 5,570 ± 30 yr and has been normalised to account for isotopic fractionation effects with respect to a ~25% enrichment on the PDB limestone scale. The two results are scarcely significantly different and there is therefore little likelihood of isotopic replacement having taken place after deposition (R. E. G. Williams, personal communication).

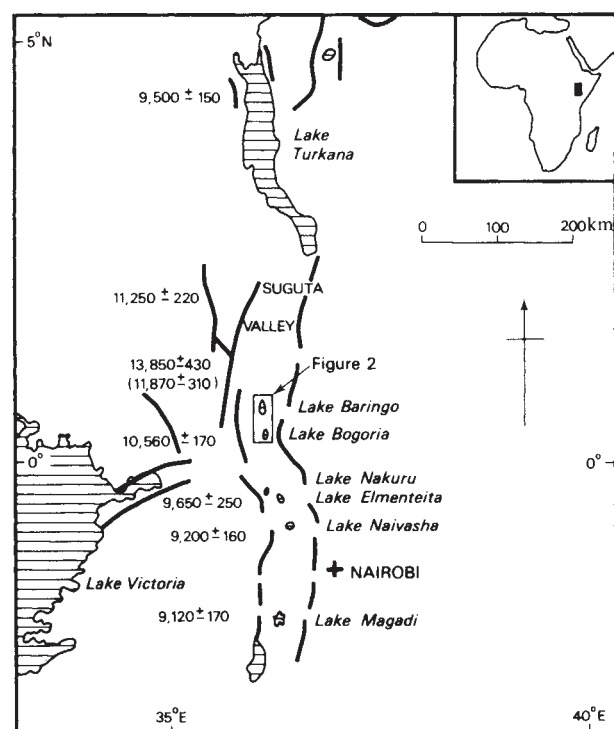


Fig. 1 Lakes of the Kenya Rift Valley. Radiocarbon dates (yr BP) of maximum Holocene extension of the lakes are indicated to the west (left) of the individual lakes or locations.

The possibility of a greater Lake Baringo that may at times have included Lake Bogoria has been discussed frequently^{8–9,13–17}. Fluvio-lacustrine Lobo Silts, which form the bulk of the sedimentary mass of the Lobo Plain have traditionally been considered of Holocene age⁸. Bogoria Silts, which are deposits of a later transgression, have recently been recognised overlying Lobo Silts in the northern Bogoria basin¹⁷. They may correlate with the early Holocene Kokwob Formation^{16,18,19} of the northern Baringo Basin, thus making the Lobo Silts of late Pleistocene age¹⁷. Whichever interpretation is correct, it has been unclear whether only one early Holocene lake existed ('Kobwob Lake') or whether the two lakes rose and fell independently of each other^{9,13,16,17}.

Radiocarbon dates for samples of *M. tuberculata* collected from a series of strandlines up to 15.85 m above the 1973 level

of Lake Baringo provide evidence of a more extended lake between 10,000 and 14,000 yr BP (Birm-541, 542, 544, 545) (Fig. 2). Shells from the highest strandline on the western margin of Lake Baringo yielded dates of $13,670 \pm 320$ yr BP (inner fraction) and $13,850 \pm 430$ yr BP (middle fraction) (Birm-542). In contrast, dates of $11,870 \pm 310$ yr BP (inner fraction) and $10,860 \pm 280$ yr BP (middle fraction) were obtained for shells from a strandline at the same height (W. W. Bishop, personal communication) immediately north of the Bogoria/Baringo watershed (Birm-541). There is no evidence of Holocene faulting in the area; and unless a lake which attained the same

maximum at different times or fluctuated around that maximum for $\sim 2,000$ yr is proposed, one of the dates seems to be spurious. The Birm-544 and 545 dates were obtained for shell samples from lacustrine sediments in lower locations, 15.54 m and 10.06 m respectively above the level of Lake Baringo.

The Birmingham dates for the Baringo and Bogoria basins are generally $\sim 1,000$ yr older than those obtained for the other Kenya Rift Valley lakes (Fig. 1). Isotopic replacement seems to have occurred in the samples from Lake Baringo, resulting in discrepancies between middle and inner shell fractions (W. W. Bishop, personal communication), and in both basins, there is the possibility of hard water effects due to ancient CO_2 derived from hot spring waters and some of the lake water bicarbonate could have been derived from alkaline lavas.

Complete correlation between the two lakes remains impossible because of a lack of accurate inter-basin altimetric evidence and conflict in the presently available heights of key locations in the two basins. The most recently available heights of the surface of Lake Bogoria and Lake Baringo are 989.5 m (ref. 10) and 967.0 m (ref. 13) respectively. Both lakes are subject to seasonal and longer-term fluctuations of several metres. The height of the relatively inaccessible watershed between the lakes is of critical importance and we suggest that it may lie at ~ 10 m above the 1976 level of Lake Bogoria, which is intermediate to two other recent estimates^{13,17}. Holocene tectonic activity is not considered likely although a northward tilt of the basin floor may have occurred^{9,15}. Whilst not resolving the problem of the existence of a single combined Kokwob Lake, the new date suggests high stands of both lakes at a period $\sim 10,500$ yr BP and makes a single large lake, or an extension of the intervening swamp a considered possibility. The relationship of the +11 m limestone to the early Holocene lake levels remains uncertain.

The range of dates for maximum extension of the lakes in the Kenya Rift Valley may reflect differences in the topographical and hydrological properties of each basin, as well as difficulties in comparing directly radiocarbon dates from different laboratories. In addition, the approximately linear north to south decrease in the dates (Fig. 1) may be partly explained by early Holocene regional climatic variations between 2°N and 2°S .

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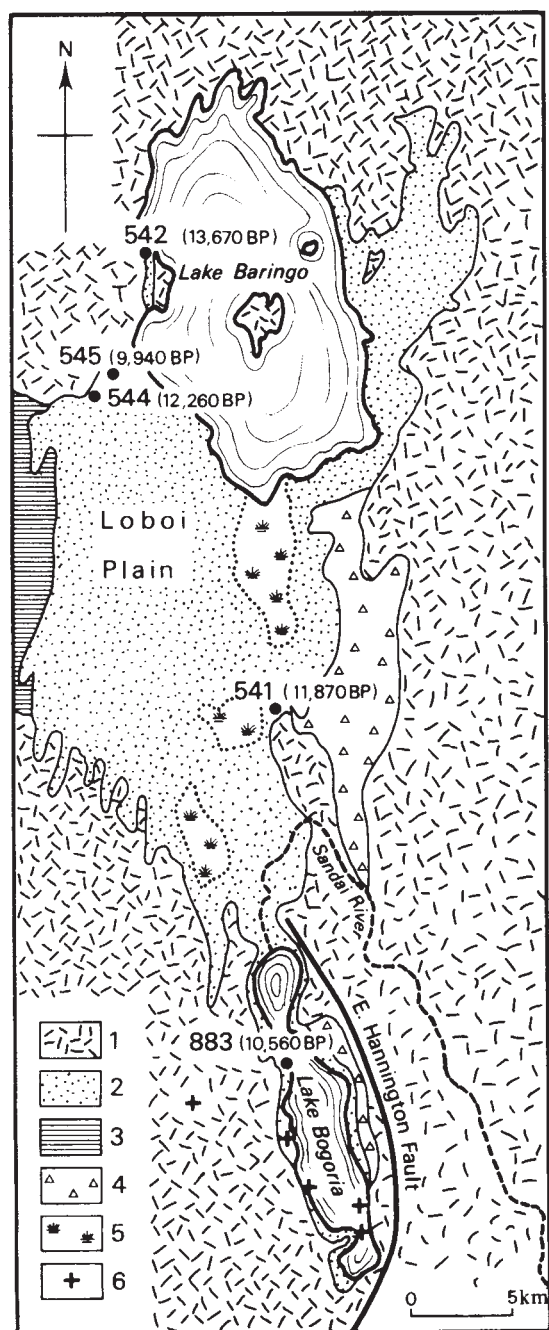


Fig. 2 Simplified geological setting of the Baringo-Bogoria basin 1, Miocene-Pleistocene lava uplands; 2, fluvio-lacustrine sediments; 3, silts and gravels of Pleistocene Kapthurin Formation; 4, fanglomerate; 5, swamp; 6, hot springs. Radiocarbon dated localities are indicated by their (Birm-) numerical prefix and the dates for the inner shell fraction are shown in parentheses.

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Phytoplankton growth and cyclonic eddies

AROUND the British Isles thermal fronts¹ develop during the summer months between cold, well mixed waters that are relatively rich in inorganic nutrients and warm, well stratified waters that are nutrient depleted². The surface standing crop of phytoplankton is generally high in the marginally stratified frontal zone, particularly where the water is deep (for example, off Ushant and around Orkney and Shetland)², although these surface waters are generally low in inorganic nutrients. To

Table 1 Surface (2m) hydrographic data and phytoplankton cell counts for stations representative of stratified, frontal and mixed waters (the station positions are shown in Figs 1 and 4a)

Station	Stratified W	Frontal X	Mixed Y	Mixed Z
Temperature (°C)	16.42	14.53	13.02	12.82
Salinity (‰)	35.22	35.25	35.26	35.26
Nitrate ($\mu\text{g at } 1^{-1}\text{-N}$)	1.4	1.7	1.9	3.8
Silicate ($\mu\text{g at } 1^{-1}\text{-Si}$)	1.3	1.3	1.6	1.8
Chlorophyll <i>a</i> (mg m^{-3})	0.55	2.25	7.22	0.81
Phaeopigment (mg m^{-3})	0.11	0.13	0.33	0.07
Diatoms (cells ml^{-1})				
<i>Chaetoceros</i> spp.*	+	164	23	59
<i>Nitzschia</i> spp.†	8	51	16	63
<i>Rhizosolenia delicatula</i> Cleve	0	8	3	10
<i>Thalassionema nitzschoides</i>	0	6	1	1
Hust.				
Dinoflagellates (cells ml^{-1})				
<i>Gyrodinium aureolum</i> Hulburt	0	6	169	5
<i>Prorocentrum micans</i> Ehrenb.	3	13	5	1
<i>Scrippsiella trochoidea</i> (Stein)	+	8	8	+
Loeblich III				

* Mainly *C. danicum* Cleve and *C. laciniosum* Schütt.

† Mainly *N. delicatissima* Cleve and *N. seriata* Cleve.

understand the growth of these frontal blooms, therefore, the scales³ of non-tidal motion that determine the exchange of chemical and biological properties in frontal regions need to be identified. Although the frontal boundaries are almost in geostrophic balance, they are unstable and are characterised by cyclonic eddies with a spatial extent of 20–40 km which can persist for a few days⁴. These eddies probably have an important influence on phytoplankton growth through the transfer of nutrients as well as phytoplankton across the frontal zone. Furthermore, as the division time of the plant cells is relatively short (~1 d), growth of the population may actually occur in the eddy transfer process. In this letter a series of observations on the Ushant frontal system, planned to investigate this problem, are described.

An infrared satellite image (Fig. 1), taken on 14 July 1978, shows a series of eddies in the frontal region between the cold, well mixed waters close to the coast of France and the warm, well stratified waters of the Celtic Sea (such baroclinic eddies are comparable with the well-known atmospheric cyclones that occur in the westerlies on a one-hundred times larger scale). The area defined by the inset was chosen for detailed study (Fig. 2) after a preliminary hydrographic survey (13–14 July) had indicated that eddy formation was most likely in this region and, accordingly, a current meter mooring was laid at station 038.

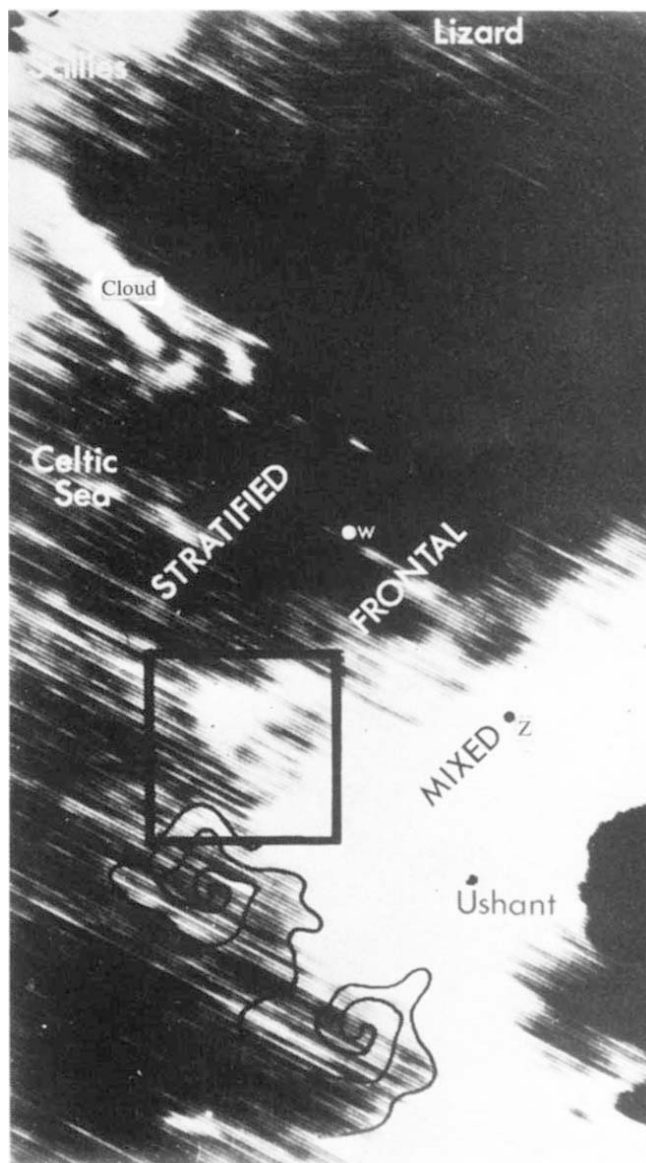


Fig. 1 Infrared satellite image of the southwestern approaches to the English Channel, taken at 10.40 GMT on 14 July 1978. Mooring 038 was located at 48°42' 75" N, 05°43' 85" W (see Fig. 2a). The inset outlines the area shown in Figs 2 and 4, and stations W and Z are referred to in Table 1.

These current records did not reveal eddy motions (Fig. 3a) but allow tidal corrections to be made to plots of the ship's track.

The surface distributions of temperature, salinity and chlorophyll *a* all reflect the cyclonic eddy structure seen in the infrared image (Fig. 2) and, as expected, the temperature profiles (Fig. 3b) showed that the cold arm of the eddy was less stratified than the adjacent warmer water. This detailed survey

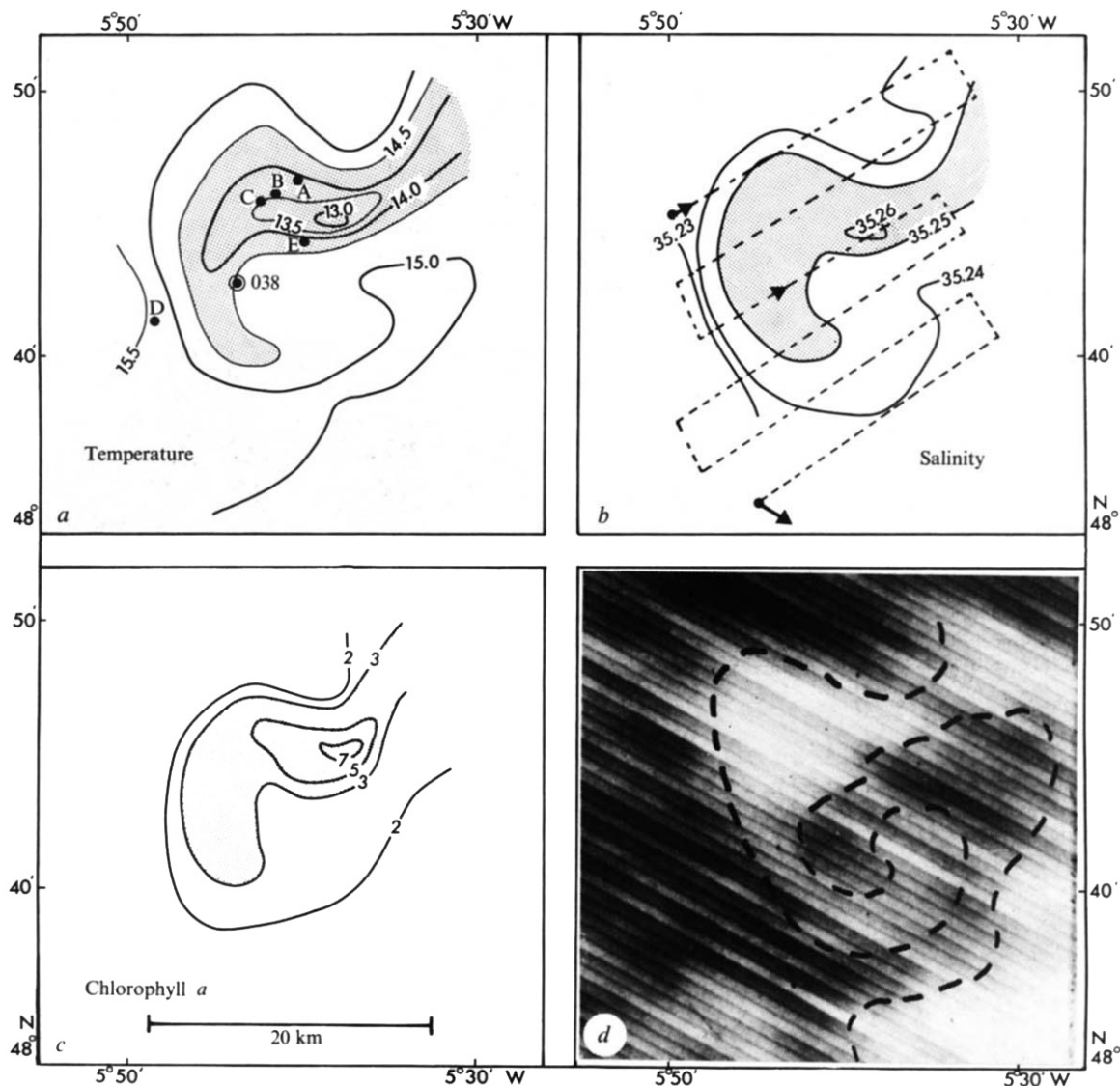


Fig. 2 *a, b, c*, Surface (2m) distributions of temperature ($^{\circ}\text{C}$), salinity (‰) and chlorophyll *a* (mg m^{-3}) between 18.30, 14 July and 03.20 GMT 15 July 1978. *d*, Infrared satellite image, enlarged from Fig. 1. The ship's track, shown in (*b*), was corrected for tidal displacement before contouring the hydrographic data. Expendable bathythermograph (XBT) casts were made at positions A–E (see Fig. 3*b*).

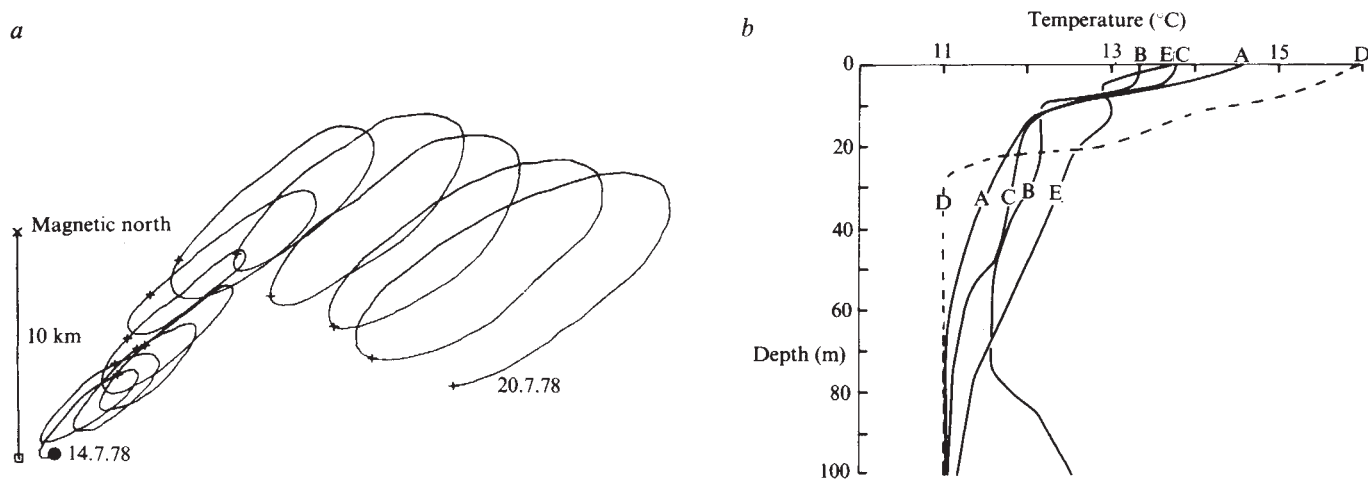


Fig. 3 *a*, Current records at 15m (98m off bottom) from mooring 038 (see Figs 1 and 2). The mooring was laid during neap tides and recovered during spring tides. The crosses mark the midnight and midday time intervals. *b*, XBT records at positions A–E (see Fig. 2*a*). The discrepancies in surface temperature shown here and in Fig. 2*a* are due to differences between 0 and 2m and the inherent smoothing effects of the deck bucket system used for continuous measurements whilst steaming (see ref. 7).

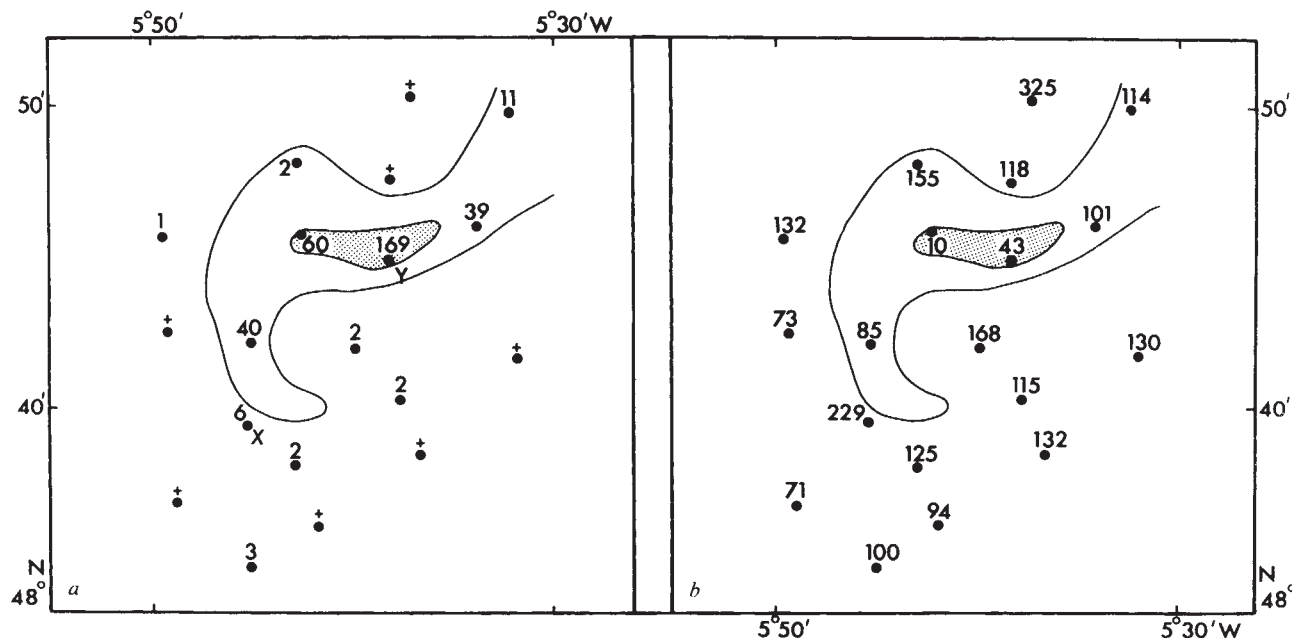


Fig. 4 Surface (2m) cell counts (ml^{-1}) for a, *Gyrodinium aureolum* and b, the diatom species listed in Table 1. (+) indicates <1 cell ml^{-1} . The position of the eddy is marked by the 14.5° and 13.5° temperature contours.

was repeated daily between 17 and 20 July, but over this period the surface temperature structure gradually became more diffuse and no new eddy structures were found, or could be detected subsequently by examination of satellite images. Surface chlorophyll *a* levels remained very variable, with high values (up to 20 mg m^{-3}) associated with horizontal temperature gradients as well as with temperature minima.

The data presented in Fig. 2 give the first confirmation of the suggestion that eddy structures seen on satellite pictures are intrusions of cold, mixed water into warmer, more stratified water. However, the coldest, saltiest region was isolated within the spiral arm of the eddy, suggesting that some of this water may have had a more local origin through upward displacement of the thermocline. The strongest evidence in support of this hypothesis comes from phytoplankton cell counts (Fig. 4) which show that the dominant organism in the cold water patch was *Gyrodinium aureolum* Hulbert, a dinoflagellate characteristic of chlorophyll-rich layers in shallow thermoclines⁵. (Vertical profiles close to the position of the eddy gave sub-surface chlorophyll *a* concentrations and cell counts for *G. aureolum* as high as 200 mg m^{-3} and 10^4 ml^{-1} respectively.) In contrast, diatoms which persist in the well mixed waters throughout the summer⁵ were relatively scarce within this cold water patch.

The distributions of phytoplankton species and inorganic nutrients across the frontal region are summarised in Table 1. It seems that the higher chlorophyll levels in water of frontal properties were due partly to a general increase in the abundance of several diatom and dinoflagellate species and partly to localised patches of *Gyrodinium aureolum* derived from the thermocline. The latter may initiate through a 'seeding' effect the development of widespread blooms of *G. aureolum* that have been observed occasionally in previous summers in the western English Channel⁶. Further experimental studies at sea are now required to establish quantitatively whether mixing of nutrients across frontal regions, due to cyclonic eddies, has a significant effect on annual primary production.

We have been concerned primarily with the influence of eddies on the transfer and growth of phytoplankton in the frontal regions around the British Isles³. The ideas developed here are also important in understanding the cross-frontal transfer and dispersal of other properties, such as heat, radioactive material, hydrocarbons and industrial wastes which are now causing much public concern in our heavily exploited shelf seas.

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Flight capability and the pectoral girdle of *Archaeopteryx*

AS the earliest birds known, the late Jurassic specimens of *Archaeopteryx* have been the object of great speculative interest. Ostrom^{1–3} has argued that *Archaeopteryx* was a terrestrial, cursorial predator that represents a preflight stage in the origin of birds in which the forelimbs were used as nets to trap insects. So far, this has been challenged mainly on the grounds that such activity would have caused excessive feather wear⁴. The principal evidence for regarding *Archaeopteryx* as flightless, or at best an inept non-flapping glider, has come from interpretations of the structure of the pectoral girdle. The absence of an ossified sternum for attachment of flight muscles has long been cited. More recently, it has been argued that the structure of the coracoid of *Archaeopteryx* would not have permitted the supracoracoideus muscle to function as a wing elevator^{2,5}. Because the asymmetrical remiges of *Archaeopteryx* prove that

the wing had an aerodynamic function⁶, we now hope to show that neither of the preceding points precludes a capacity for powered flight in *Archaeopteryx*.

There are several generally held misconceptions concerning the pectoral girdle of modern birds. The most prevalent of these is that the carina of the sternum is the principal site of origin of the massive pectoralis muscle, which provides the power stroke of the wing. This is not so. In most birds, *m. pectoralis* originates to a greater extent from the furcula and the coraco-clavicular membrane (Fig. 1). As it passes posteriorly its fibres originate on the sternum only from those areas not pre-empted by the underlying *m. supracoracoideus*. Typically, these areas consist of a narrow band on the ventral margin of the carina and the most lateral and posterior portions of the sternal plate (Fig. 2). In such birds as the *Dendrocolaptidae*, in which the carina is reduced to facilitate tree-trunk foraging⁷, the pectoralis muscle becomes thin and broad, spreading out laterally and dorsally well past the sternum and on to the rib cage. Once anchored to the furcular area, it would seem that *m. pectoralis* could expand posteriorly and attach to any underlying structure that happened to be present.

Apart from feathers, the character of *Archaeopteryx* most often cited as being bird-like is the well developed furcula. Relative to modern birds of the same size, the furcula of *Archaeopteryx* is actually hypertrophied. There has been no satisfactory explanation of this structure which has received mostly perfunctory treatment. Ostrom merely asks: "Did it function as a transverse spacer between the shoulder sockets?"² Why such a structure would be needed in *Archaeopteryx* and not in any of its suggested ancestors is never dealt with. We consider that the extremely robust furcula of *Archaeopteryx* is best interpreted as having been the site of origin of a well developed pectoralis muscle.

When it is observed that *m. pectoralis* arises to a large extent from the furcular area, it can then be seen that the main function of the ossified sternum and carina in modern birds is to provide attachment for the supracoracoideus muscle (Fig. 2). Although the belly of *m. supracoracoideus* is situated ventrally, it serves to raise the wing because its tendon passes above the glenoid facet and over the acrocoracoid process of the coracoid to insert on the dorsal aspect of the humerus (Fig. 2). In modern birds it is the largest of the muscles that effect the recovery stroke of the wing. For this reason, Ostrom² has emphasised the absence of an acrocoracoid process in *Archaeopteryx*, suggesting that if *m. supracoracoideus* had been present it could not have functioned to raise the wing. Ostrom interprets this as evidence for *Archaeopteryx* having been flightless, the implication being that *m. supracoracoideus* is essential for flight. That this is not the case has been conclusively proven by Sy⁸, who cut the supracoracoideus tendons in living examples of crows (*Corvus*) and pigeons (*Columba*) and found that the birds were still capable of normal, sustained flight. It is of considerable interest that the only capacity lost was the ability of the pigeon to take off from level ground.

It has thus been proved that in modern birds the dorsal elevators (principally *m. deltoideus major*⁸) are completely capable of effecting the recovery stroke of the wing. These

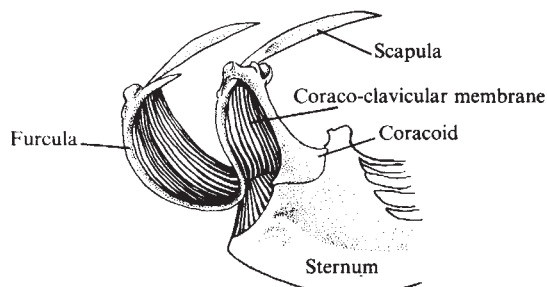


Fig. 1 Left side of pectoral girdle of the duck *Bucephala clangula* showing the furcula and extensive coraco-clavicular membrane from which *m. pectoralis* has its main origin (redrawn from Sy⁸).

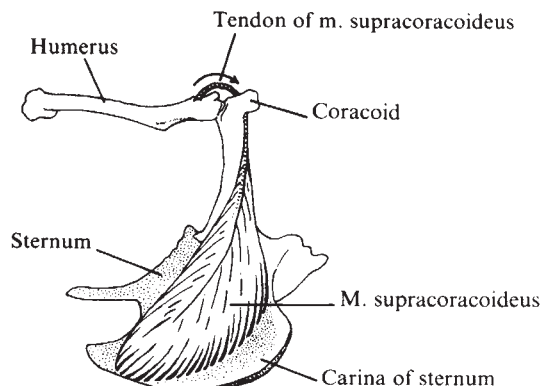


Fig. 2 Right side of pectoral girdle of the pigeon *Columba livia* to show the action of *m. supracoracoideus* and the extensive area of the sternum that this muscle occupies. *M. pectoralis* attaches to the sternum only on those areas shown in stipple. (Modified from Ostrom², after George and Berger¹⁰.)

muscles originate mainly from the scapula, which in *Archaeopteryx* forms an acute angle with the coracoid, as is true in adults of modern flying birds. The acute angle shortens the distance through which the dorsal elevators must act, thus giving greater power. In most flightless birds, on the other hand, the acute angle is lost and the scapula is more nearly vertical⁹.

The dorsal musculature is present and is used to raise the forelimb in virtually all vertebrates; furthermore, it is the dorsal musculature that elevates the wing in such volant forms as bats. Therefore, it is logical to assume that in the evolution of avian flight the recovery stroke of the wing would first have been carried out by the dorsal elevators. The enlarged, ventrally situated *m. supracoracoideus*, the acrocoracoid process, and the ossified sternum with a keel, constitute a single functional complex that is not a requisite of flight but merely a refinement that was superimposed in later birds on an apparatus probably already capable of full flight. The enlarged *m. supracoracoideus* probably evolved to counter the action of an increasingly enlarged *m. pectoralis* and, as evidenced by Sy's experimental pigeons, may have been necessary for birds to adopt purely terrestrial habits.

In conclusion, the robust furcula of *Archaeopteryx* would have provided a suitable point of origin for a well developed pectoralis muscle. Furthermore, the supracoracoideus muscle, and hence an ossified sternum, is not necessary to effect the recovery stroke of the wing. Thus the main evidence for *Archaeopteryx* having been a terrestrial, cursorial predator is invalidated. There is nothing in the structure of the pectoral girdle of *Archaeopteryx* that would preclude its having been a powered flier.

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A new kind of neural superposition eye: the compound eye of male *Bibionidae*

IN neural superposition eyes the signals of receptors pointing to one region in space are neurally pooled. The optical and neural organisation of these eyes yields an enhanced absolute light sensitivity while minimising the loss of visual acuity that accompanies pooling. In the compound eyes of flies (Diptera), this is achieved in the following way. The distal endings of seven retinula cells lie near the back focal plane of each lens¹. Their light-sensitive structures, the rhabdomeres, are optically isolated from each other. The rhabdomeres of one ommatidium have different optical axes. The optical axes of seven rhabdomeres in seven adjacent ommatidia are parallel (Fig. 1a, ref. 2). The axons of such retinula cells (except the central ones, which pass through to the second optic ganglion) converge onto the same cartridge of secondary neurones in the first optic ganglion, the lamina^{3,4}. Thus, each lamina cartridge 'looks at' one point in space. In one suborder of flies (Cyclorrhapha), to which the housefly *Musca* and the fruitfly *Drosophila* belong, the arrangement of rhabdomeres is asymmetrical (Fig. 1a). Eyes organised in this way are one form of neural superposition eyes². Here, I provide evidence that a different kind of neural superposition eye exists in the *Bibionidae*, a family of flies of the suborder Nematocera. *Bibionids* are peculiar in being extremely sexually dimorphic in their visual system: the males have, in addition to small 'female' eyes, a pair of large dorsal compound eyes, functionally correlated presumably with the male habit of swarming and chasing females. These dorsal eyes differ from the eyes described in more advanced flies in two respects: the rhabdomeres are symmetrically arranged and the rhabdomeres with parallel optical axes are found in ommatidia $\sqrt{3}$ times further apart than adjacent ommatidia. The course of the retinula cell axons in the lamina differs accordingly.

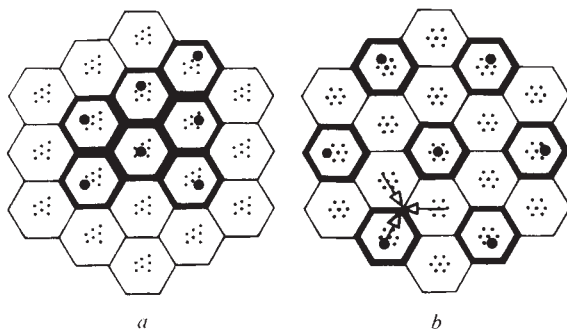


Fig. 1 Arrangement of rhabdomeres and optical organisation of the compound eyes of *a*, cyclorrhaphan flies (after Kirschfeld²) and, *b*, nematoceran flies (*Bibionidae*). Rhabdomeres marked with large dots have parallel optical axes. Arrows in *b* show the initial course of retinula cell axons in the lamina, as predicted from optical data.

The technique of antidromic illumination⁵ was used to investigate the optical organisation of the eye. The flies are immobilised with beeswax, and light shone into the ventral side of the head, where a small window has been cut into the cuticle. In this experimental situation, light is diffusely scattered inside the head and travels antidromically up the rhabdomeres, which function as light guides. The distal tips of the rhabdomeres thus become light sources in the back focal plane of each lens. Real and virtual images of the rhabdomere tips can be observed with a microscope, focused at different levels above and below the cornea. Anatomical data were derived from reduced silver preparations⁶.

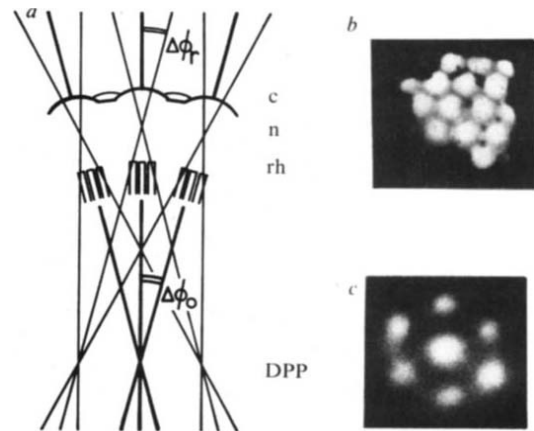


Fig. 2 *a*, Geometrical construction to show the relationship between size and depth of the deep pseudopupil (DPP), the inter-ommatidial angle, $\Delta\phi_0$, and the angle between rhabdomeres, $\Delta\phi_r$. *c*, Cornea; *n*, nodal point; *rh*, distal tips of rhabdomeres. Not drawn to scale. Histological data for *Bibio marci* are as follows (after correction for 15% shrinkage). Radius of curvature of lens, 23.1 μm ; distance of rhabdomere tips from the lens surface, 82.8 μm ; separation of rhabdomere tips, 3.0 μm . The angle subtended by the rhabdomeres at the nodal point is 2.88°, assuming the nodal point to lie in the centre of curvature of the lens (this is the case when the air-cuticle interface alone provides the refractive power of the lens). This agrees well with the optical data listed below. *b*, Pattern of lenses at the level of the cornea. Except for the area shown, the eye is covered with black ink to reduce stray light. Mean distance of adjacent facets, 32.96 μm . The inter-ommatidial angle is given by $\tan \Delta\phi_0 = 32.96/1,100$. $\Delta\phi_0 = 1.72^\circ$. The separation of facets $\sqrt{3}$ times further apart (like those marked in Fig. 1*b*), 55.86 μm . The angle, $\Delta\phi'_0$, between these facets is 2.91°. *c*, Deep pseudopupil. Superposition of virtual images of the distal tips of rhabdomeres at the centre of local curvature of the eye, 1,100 μm below the cornea. Mean distance of the peripheral images from the central one, 55.48 μm . The angle between rhabdomeres is given by $\tan \Delta\phi_r = 55.48/1,100$. $\Delta\phi_r = 2.89^\circ$. Photographs taken through a Leitz Orthoplan microscope, antidromic illumination, male *Bibio marci*.

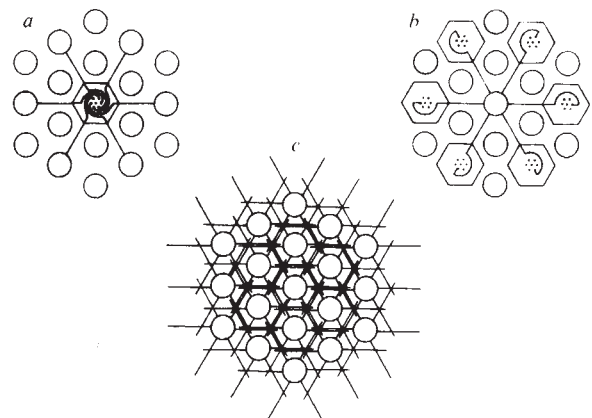


Fig. 3 Hypothesised course and distribution of retinula cell connections in the lamina of male bibionids. Circles represent horizontal views of cartridges. *a*, Divergence of axons from one ommatidium to six different cartridges, corresponding to six different optical axes. Note that the axons have to turn $\sim 180^\circ$ and that they have to pass between two neighbouring cartridges to reach the appropriate one. *b*, Convergence of axons transmitting information about the same region in space, that is, axons of retinula cells having parallel optical axes. Note that these converging axons take the same routes as the divergent ones. *c*, Divergent and convergent axons drawn in one diagram. The fibres form a hexagonal lattice with the same orientation as the corneal lattice of lenses. Histological examination (Fig. 4) indicates that it is not the axons, but their collaterals, that form this network of connections.

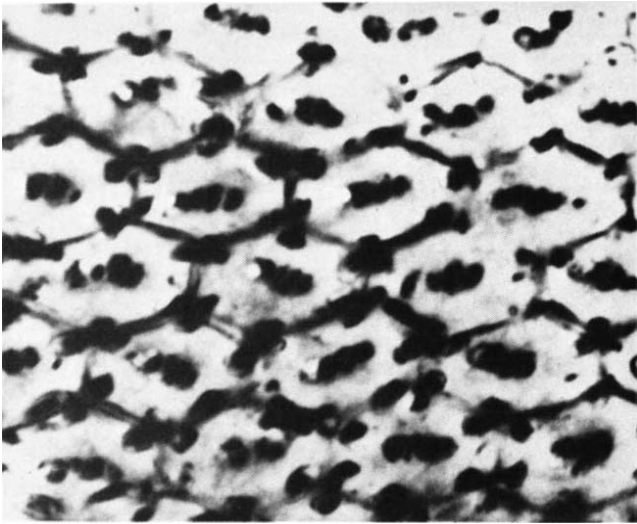


Fig. 4 Transverse section through the lamina, showing the network of axon collaterals of retinula cell axons. Triads in the corners of the lattice are profiles of main axons running perpendicular to the plane of sectioning down into the lamina. The pattern of fibres is strikingly different from that seen in other flies^{3,4}. Bar, 10 μ m; Bodian silver; *Bibio marci*.

The optical axes of the ommatidia (that is, of their central rhabdomeres) intersect at the centre of curvature of the eye (Fig. 2a). At this point (1,100 μ m beneath the cornea in the case of the male eye of *Bibio marci*) the microscope visualises an enlarged image of the receptor endings, originating from the superposition of virtual images of the rhabdomere tips thrown by each lens along its optical axis (Fig. 2b). This image has been termed the deep pseudopupil (DPP, refs 7, 8). The distance of the DPP from the cornea is determined by the inter-ommatidial angle, $\Delta\phi_0$, and its size is a function of the inter-rhabdomere angle, $\Delta\phi_r$ (Fig. 2a, refs 7, 8). In bibionids, which have a symmetrical arrangement of rhabdomeres typical of nematoceran flies^{9,10}, the size of the DPP exactly matches the size of the lens pattern shown in Fig. 1b (Fig. 2b). This means that for the arrangement of ommatidia marked in Fig. 1b the inter-ommatidial angle, $\Delta\phi'_0$ (which is $\sqrt{3}$ times the angle, $\Delta\phi_0$, between adjacent ommatidia), is equal to the angle between rhabdomeres. Every seven rhabdomeres, having relative positions like those marked in Fig. 1b, point in the same direction in space. It follows that the optical requirements of a neural superposition eye are fulfilled.

It remains to be shown, however, that the neural conditions are met: that is, that the axons of retinula cells with parallel optical axes converge onto the same cartridge (set of secondary neurones) in the lamina. Given the optical organisation outlined above, a hypothesis can be advanced about the anatomical organisation of the retina-lamina projection (Fig. 3). Two outstanding and easily testable features of the projection emerge from this hypothesis: (1) to reach their respective cartridges, the axons of the six peripheral retinula cells in one ommatidium have to twist through 180° and diverge in opposite directions, and (2) the axons or their collaterals have to pass between cartridges, forming a hexagonal network.

In horizontal histological sections the axons of the retinula cells, leaving the retina in tight bundles, can be seen to rotate about 180° on entering the lamina. In crossing the superficial layer of cell bodies of lamina neurones, the axon bundles open up. This brings three axons of three adjacent ommatidia into close proximity (see Fig. 1b). The clusters of three axons run down into the lamina, parallel to the long axes of the cartridges, marking the corners of a hexagonal lattice in horizontal sections. In the course of running down into the lamina, the axons give off collaterals, which pass between adjacent cartridges towards the ones in the next row. These collaterals form a hexagonal

network of fibres which have the same orientation as the corneal pattern of hexagonal lenses (Fig. 4). Therefore, it seems to be these collaterals which establish connections with the appropriate cartridges. At the level of light microscopy, the anatomical structure of the lamina therefore agrees with the predictions made, based on the optical structure of the retina.

Up to now neural superposition eyes have been found in only one other order of insects. Although in this case (Hemiptera¹¹) the retina-lamina projection has not been described in detail, the optical organisation is quite different from that of dipteran eyes. The rhabdomeres with parallel optical axes lie in ommatidia even further apart (3–4 facets) than in the case of the nematoceran flies described here. This raises several questions about the evolution of neural superposition eyes. Within Diptera, at least, we may ask what functional and/or structural reasons might have caused the topological rearrangement from the symmetrical, widely spaced organisation of a nematoceran eye to the asymmetrical one of flies considered to be more advanced in evolution.

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The role of inner hair cells in hearing

DISCUSSION about functional differences between the inner and outer hair cells (IHCs and OHCs) in the mammalian organ of Corti^{1–5}, has arisen as a result of several anatomical and electrophysiological observations. The proportions of afferent and efferent fibres supplying the two types of cells are quite different^{6–8}; the afferent neurones associated with them are distinct cytologically as well as in their susceptibility to retrograde degeneration^{6–8}; the electrophysiological responses following preferential loss of OHCs are not what would be expected on the basis of a similarity of function^{2,5,9–11}. Attempts at the elucidation of these differences^{2,9} have made use of the ototoxic antibiotic kanamycin, which causes preferential damage to OHCs. This technique is far from ideal: the damage may be patchy and IHCs may also be affected. As to the selective elimination of IHCs, no procedure has been available. Mutant genes frequently provide material that cannot be produced experimentally, but although a large number of genes affecting the inner ear in various ways are known in the mouse^{12–14}, there

has been none so far that clearly discriminated between IHCs and OHCs. We now report a new mutation in the mouse which selectively eliminates IHCs. As the mutant animals are almost totally deaf, it would seem that hearing is predominantly dependent on IHCs.

The new mutation occurred at Albert Einstein College of Medicine, Bronx. It is an autosomal recessive gene with full penetrance. The homozygotes show an abnormality of behaviour that is known as the waltzing syndrome¹², and to conform with general usage the gene has been named *bronx waltzer* and assigned the symbol *bv*. The hearing ability of *bv/bv* mice is severely impaired. The affected animals either give no response to sound at all or respond very slightly during a short period following the normal time of onset of hearing (at about 13 d). The hearing tests were based on Preyer's pinna reflex.

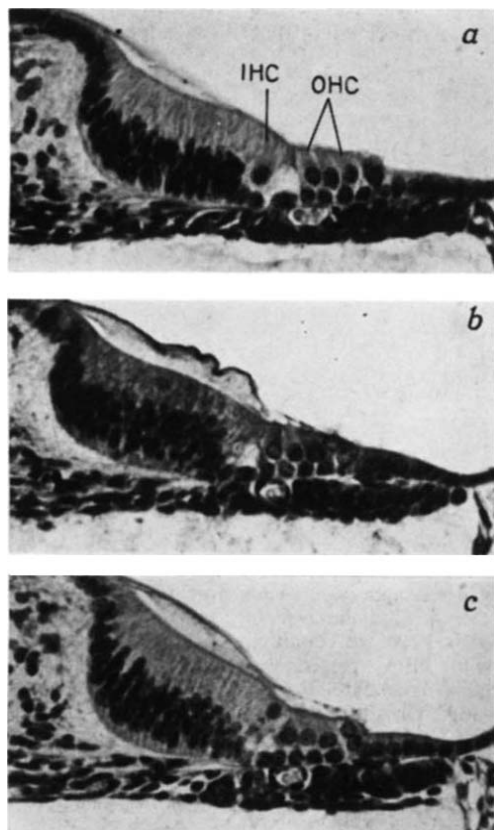


Fig. 1 Organ of Corti in newborn mice. *a*, $+/+$; IHC normal. *b*, *bv/bv*; IHC absent. *c*, *bv/bv*; IHC abnormal.

This method is far from refined, but experience has shown that it is reliable enough for preliminary studies. Moreover, the results of these tests were subsequently confirmed by the severe degeneration of the spiral ganglion, observed even in very young animals.

Altogether, 31 *bv/bv* animals, ranging in age from newborn to 281 d old, and 14 normal controls ($4+/bv$, $10+/+$), ranging from newborn to 253 d old, were used in this study. They were fixed in Witmaack's fluid by intravital perfusion, and the heads were decalcified in 1% nitric acid. The otic capsules, with the surrounding tissues intact, were embedded in celloidin, impregnated with paraffin wax, and sectioned at 10 μ m in a plane parallel to the modiolus. The sections were stained with Ehrlich's haematoxylin and orange G containing a trace of erythrosin.

The gross structure of the cochlea in *bv/bv* mice is normal. Histological abnormalities occur in the organ of Corti and the spiral ganglion. Other structures, including the stria vascularis, seem to be normal. In the organ of Corti the pillars and IHCs may be absent or malformed. As the pillars are formed well after birth, their abnormalities appear later, but those of IHCs are clearly visible even in the newborn (Fig. 1). A large proportion

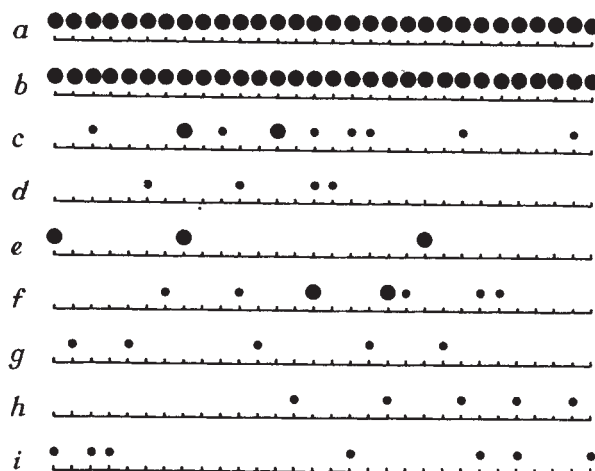


Fig. 2 Distribution of normal (large spot), abnormal (small spot) and missing (no spot) IHCs in 30 consecutive sections from the middle part of the left cochlea. *a*, $+/+$; newborn. *b*, $+/+$; 241 d old. *c*, *bv/bv*; newborn. *d*, *bv/bv*; 3 d. *e*, *bv/bv*; 6 d. *f*, *bv/bv*; 9 d. *g*, *bv/bv*; 29 d. *h*, *bv/bv*; 150 d. *i*, *bv/bv*; 244 d.

of IHCs are not formed at all, and of those that are formed the vast majority are grossly abnormal. They may be very small and rounded or they may occur in strikingly abnormal positions. There may in some cases be two abnormal cells side by side instead of a single normal one. However, occasional IHCs are observed that are normal in appearance and position. Further study would be required to determine whether they actually are normal. These apparently normal cells had a tendency to disappear with age, although some remain even in the oldest animals studied. To determine the distribution of the normal, abnormal and missing IHCs, 30 consecutive sections from the middle part of the cochlea were examined in both the ears of 12 animals ranging in age from newborn to 244 d old. Figure 2 shows the distributions observed in the left ears of a representative sample. The two sides were generally very similar.

The density of the neurones in the spiral ganglion seems to be approximately normal in the newborn, but their degeneration is so rapid that the ganglion is obviously thinner by 6 d, and after about 4 months very few cells remain in it (Fig. 3). Specialised techniques would be required to determine the type of the remaining cells and whether they are normal, but as far as the extent of the degeneration is concerned, it is consistent with Spöndlin's⁶⁻⁸ finding that in the cat >90% of all spiral ganglion cells innervate IHCs, and are subject to retrograde degeneration.

As to OHCs, some may be a little irregular in shape, and an occasional cell may even be missing, but generally they seem to be normal. It is assumed that they are also normal in function,

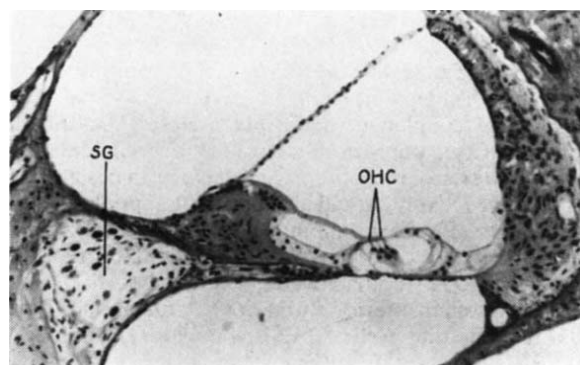


Fig. 3 Organ of Corti and spiral ganglion (SG) of a 150-d-old *bv/bv* mouse showing the absence of IHC and degeneration of SG.

not only because they do not degenerate but also because the tunnel fibres leading to them are intact even in the oldest animals.

These findings suggest that 'basic' hearing is predominantly, if not wholly, dependent on IHCs. OHCs may take part in further discriminatory processes or may even govern the IHCs in some way, but they are incapable of leading to the development of normal hearing ability on their own. Further work on this mutant using specialised techniques may lead to a clearer understanding of the functional differences between IHCs and OHCs.

This gene also affects the vestibular part of the inner ear, but this is not believed to be the cause of the 'waltzing' behaviour. Investigations on a large number of other mutants with this type of behaviour suggest that its cause should be sought in the central nervous system and not in the inner ear¹⁵.

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Dopamine inhibits adenylate cyclase in human prolactin-secreting pituitary adenomas

THERE is evidence for the existence of multiple forms of dopamine (DA) receptors¹⁻³. In particular, some of these receptors are coupled to adenylate cyclase (DA-stimulating enzyme activity), whereas others seem to be independent³⁻⁵. These two classes of receptors, which have been designated D-1 and D-2, respectively, would also have different pharmacological properties³. Pituitary mammothroph DA receptors regulating prolactin (PRL) secretion are considered to be typical D-2 receptors. A DA-stimulated adenylate cyclase has not been detected in normal anterior pituitaries^{3,5,6}; furthermore, several studies indicate that cyclic AMP is stimulatory and not inhibitory to PRL secretion^{7,8}. We report here that in homogenates of human PRL adenomas, in which dopaminergic agonists act as inhibitors of PRL secretion, basal adenylate cyclase is inhibited by DA. The DA receptors mediating this inhibition have the

same pharmacological properties as those regulating PRL secretion.

Adenomas were surgically removed from hyperprolactinaemic patients through the transnasosphenoidal route. The adenomatous nature of the surgical fragments was confirmed both by histological examination and by SDS-polyacrylamide gel electrophoretic analysis⁹ of the homogenates used for the adenylate cyclase assay. The latter revealed in all cases a prominent PRL content, whereas growth hormone, which in the electrophoretograms of normal human anterior pituitaries gives a major band (our unpublished observations), was absent. The preoperative plasma levels of PRL and the effects on these levels of dopaminergic agents are shown in Table 1. In six out of the seven cases examined, dopaminergic drugs were clearly inhibitory. Moreover, *in vitro* perfusion experiments carried out in separate adenomas revealed that such inhibition is due, at least partially, to a direct effect of DA on adenoma cells (Fig. 1).

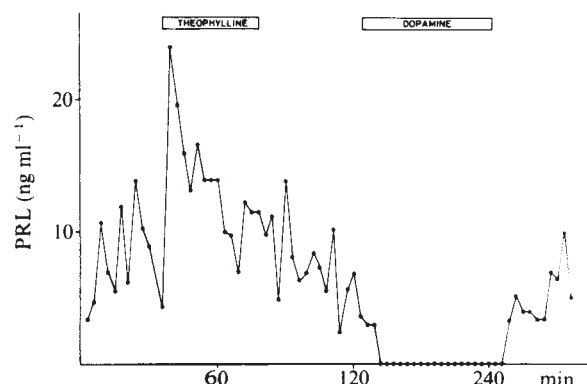


Fig. 1 *In vitro* release of PRL from a human PRL adenoma. Adenoma fragments were immersed, immediately after surgical removal, in minimum essential medium (MEM) at ice temperature and kept as such until they were mounted in a superfusion apparatus³⁰ (within 2 h of the operation). Perfusion was carried out at 37°C with MEM gassed with an oxygen/carbon dioxide (95%/5% w/v) mixture. Fractions were collected every 3 min starting at 90 min after the beginning of the superfusion. During the periods indicated by the horizontal bars the superfusion medium contained 5.5 mM theophylline or 10 μ M DA. Duplicate aliquots of the superfusate fractions were analysed for the PRL content by radioimmunoassay. Theophylline, which increases the intracellular levels of cyclic AMP through a blockade of phosphodiesterase, is stimulatory on secretion, whereas DA is strongly inhibitory. Before adenomectomy the plasma PRL concentration was 70 ng ml⁻¹ and this was reduced by more than 50% by the administration of dopaminergic drugs (see legend to Table 1). The radioimmunoassay for PRL was carried out according to Sinha *et al.*²⁸.

Table 1 also shows the effect of 10 μ M DA on basal adenylate cyclase in homogenates of individual adenomas. The inhibitory effect of DA on adenylate cyclase *in vitro* parallels the effect of dopaminergic agents on PRL secretion *in vivo*. The action of DA was dose dependent (Fig. 2a, b), with IC₅₀ values of 3×10^{-7} – 10^{-5} M in individual cases. The inhibitory effect of DA on adenylate cyclase is mimicked by the dopaminergic ergot, CH-29-717 (Sandoz) (a potent water-soluble analogue of CB-154, (ref. 10)), and by (-)-noradrenaline (NA) (Fig. 2b, c). In agreement with *in vitro* studies on PRL secretion and on binding of dopaminergic drugs to pituitary membranes carried out in animal pituitaries¹¹⁻¹³, NA is less potent than DA, but CH-29-717 is effective at even lower concentrations than DA. The specificity of the DA action was confirmed from the effects of the dopaminergic antagonists. In the responsive cases, low doses of drugs of the phenothiazine group (trifluoperazine and fluphenazine) had no effect on basal adenylate cyclase, but

Table 1 Effect of dopaminergic drugs on PRL secretion *in vivo* and of DA on basal adenylate cyclase *in vitro*

Case	PRL plasma levels before adrenalectomy		Adenylate cyclase activity		
	Basal levels (ng ml ⁻¹)	% Reduction induced by administration of dopaminergic drugs	Cyclic AMP formed (pmol per mg protein per 8 min)		% Variation induced by 10 μ M dopamine
			Control	Dopamine	
1	95	>50%	165.52 $\pm$ 13.76	111.76 $\pm$ 8.31*	-32.48
2	120	>50%	204.56 $\pm$ 17.14	144.40 $\pm$ 4.76*	-29.41
3	90	>50%	193.60 $\pm$ 1.60	118.08 $\pm$ 1.16†	-39.41
4	65	>50%	53.44 $\pm$ 0.78	45.84 $\pm$ 2.00‡	-14.22
5	72	>50%	112.48 $\pm$ 2.37	89.11 $\pm$ 7.68*	-20.77
6	120	>50%	50.79 $\pm$ 1.59	44.21 $\pm$ 0.66*	-12.95
7	200	No effect	55.69 $\pm$ 1.30	56.98 $\pm$ 1.67	No effect

Patients were infused with DA (2 μ g per kg per min $\times$ 3 h) or given CB-154 (Sandoz) (5 mg orally). Plasma levels of PRL were evaluated either at the end of the infusion, or after the CB-154 administration. For the enzyme assay, adenoma fragments were frozen to -20 °C immediately after surgical removal and thawed only just before the assay (4–15 d later). They were homogenised in 20 vol (w/v) of 2 mM Tris-maleate buffer, pH 7.4, containing 2 mM EGTA. The final incubation mixture (0.1 ml) contained: 80 mM Tris-maleate pH 7.4, 10 mM theophylline, 1 mM cyclic AMP, 0.2 mM EGTA, 0.15 mM ¹⁴C-ATP (20 mCi mmol⁻¹) (except in cases 1 and 2 where ¹⁴C-ATP was 0.5 mM), MgSO₄ (2 mM in cases 1 and 2, 0.6 mM in case 3 and 1.5 mM in all other cases), phosphocreatine (1.8 mg ml⁻¹) and creatinphosphokinase (7 U ml⁻¹). Protein concentration varied between 0.4 and 1 mg ml⁻¹. The reaction was started by the addition of the homogenate, carried out for 8 min at 30 °C and stopped by a solution containing 2% SDS, 1.4 mM cyclic AMP and 40 mM ATP. Biochemical assays were carried out according to the following procedures: adenylate cyclase, ref. 27; PRL, ref. 28; protein, ref. 29. Values for adenylate cyclase activity represent the mean $\pm$ s.e.m. of at least three replicate samples.

* $P < 0.05$; † $P < 0.001$; ‡ $P < 0.025$.

competitively antagonised the inhibitory action of DA (Fig. 2a). The DA effect was also blocked by (-)sulpiride (Ravizza), an antagonist of mammotroph DA receptors^{11,14,15}. In case 7, dopaminergic agents failed to decrease PRL plasma levels, and DA was without effect on the basal adenylate cyclase activity of the homogenate. However, in this case, adenylate cyclase was stimulated by low doses of phenothiazines¹⁶ and such stimulation was competitively counteracted by DA (Table 2). Thus, even in case 7, DA has a potential ability to inhibit adenylate cyclase.

Our results, which indicate a striking similarity between DA receptors mediating adenylate cyclase inhibition and DA receptors inhibiting PRL secretion, suggest that a decrease in the intracellular concentration of cyclic AMP might mediate the action of DA in mammotrophs. Doses required to inhibit adenylate cyclase are higher than those which are known from *in vitro* studies on animal pituitaries to be effective on PRL secretion^{12,13}; however, in other systems also, discrepancies

have often been observed between the drug concentrations which elicit physiological responses thought to be mediated by cyclic AMP, and those required to modulate the adenylate cyclase-cyclic AMP system. Several explanations have been proposed to account for these results^{17–19}. The consistency of our findings in the adenomas examined suggests that the effect of DA on adenylate cyclase is not due to a fortuitous association with a tumoral clone, but that it might be a physiological characteristic of all PRL-secreting cells. In the normal anterior pituitary the effect of DA on mammotroph adenylate cyclase might go undetected, because basal adenylate cyclase activity is contributed primarily by other cell types. Furthermore, the inhibitory coupling between DA and adenylate cyclase might be more pronounced in tumour cells. In fact, clinical data suggest the existence of a defective dopaminergic control of the pituitary in hyperprolactinaemic patients with adenomas¹⁵, and this might lead to supersensitivity of DA receptors in adenomatous cells²⁰.

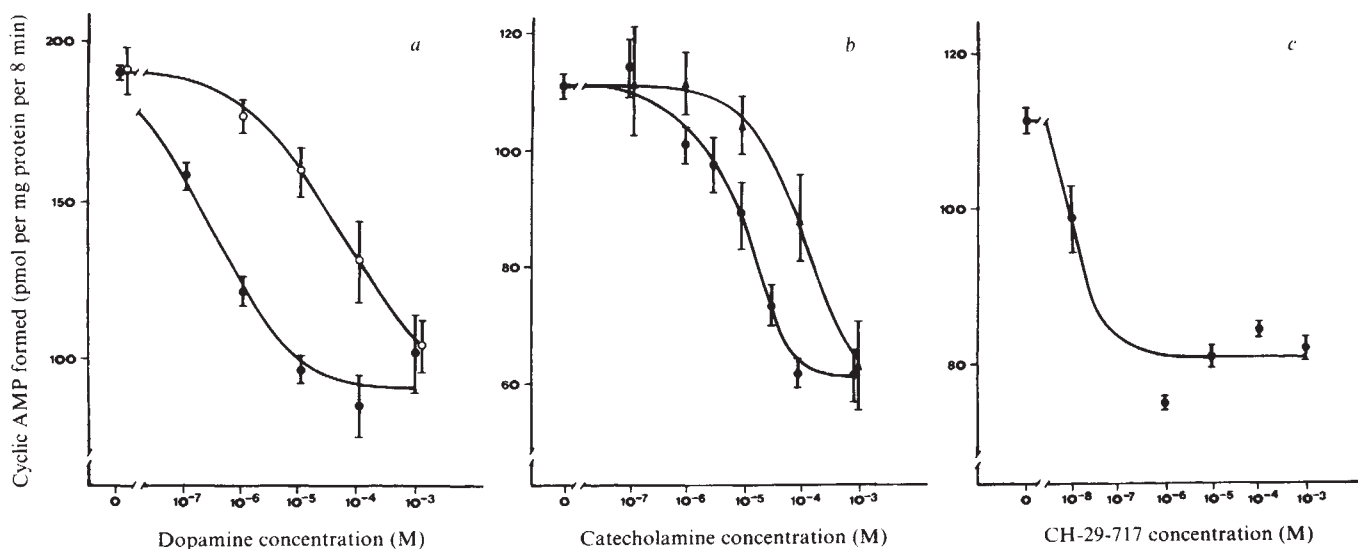


Fig. 2 Effect of drugs on adenylate cyclase activity in homogenates of human PRL adenomas. *a*, Effect of various concentrations of DA in the absence (●) and presence (○) of 0.1 μ M trifluoperazine (Maggioni) (case 3). *b*, Effect of various concentrations of DA (●) and (-)noradrenaline (▲) (case 5). *c*, Effect of various concentrations of the dopaminergic ergot CH-29-717 (Sandoz) (case 5). See legend to Table 1 for experimental details. Values represent the mean $\pm$ s.e.m. of at least three replicate samples.

Table 2 Case 7: the stimulation of adenylate cyclase by fluphenazine is competitively counteracted by DA

Addition	Cyclic AMP formed (pmol per mg protein per 8 min)
Control	65.84 ± 3.58
Fluphenazine 0.1 µM	82.32 ± 2.72*
Fluphenazine 0.1 µM + DA 10 ⁻⁶ M	77.21 ± 2.66
Fluphenazine 0.1 µM + DA 10 ⁻⁵ M	70.20 ± 1.75
Fluphenazine 0.1 µM + DA 10 ⁻⁴ M	66.24 ± 3.59
Fluphenazine 0.1 µM + DA 10 ⁻³ M	64.78 ± 1.79†

See legend to Table 1 for experimental details. Values represent the mean ± s.e.m. of at least three replicate samples.

* $P < 0.005$ relative to control; † not significant relative to control and $P < 0.001$ relative to fluphenazine (Squibb) 0.1 µM.

Note that at another site where pharmacological evidence indicates the existence of D-2 DA receptors, such as on central dopaminergic terminals²¹⁻²³, cyclic AMP and DA produce opposite effects, the former being stimulatory and the latter inhibitory to the synthesis of the neurotransmitter²⁴⁻²⁶. This suggests that an inhibitory coupling with adenylate cyclase might be a more general property of D-2 DA receptors.

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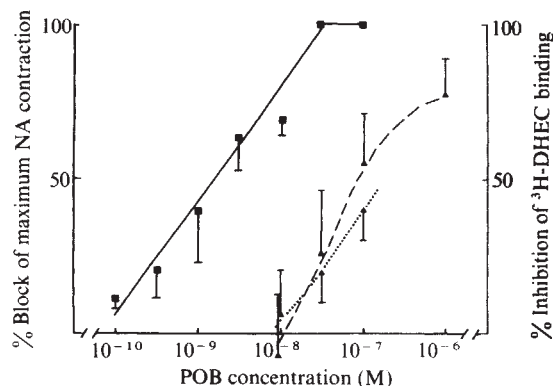
Dihydroergocryptine binding and α -adrenoreceptors in smooth muscle

DIHYDROERGOCRYPTINE is a potent antagonist of α -adrenoreceptors¹ that has recently been labelled to high specific radioactivity with tritium². ³H-DHEC was found to bind to a crude membrane preparation of rabbit uterus, and a portion of this binding was suppressible by phentolamine². The saturability, reversibility and structural and steric selectivity of this specific binding component suggested that it represents α -adrenoreceptors mediating uterine contractions²⁻⁴. In subsequent studies, ³H-DHEC was used to tentatively identify α -adrenoreceptors mediating various biological responses in several tissues⁵⁻¹⁰. The evidence for the identity of ³H-DHEC binding sites in tissue fragments with functional α -receptors in intact tissues is indirect, and is based on the demonstration of similar potencies of various ligands in suppressing ³H-DHEC binding and producing or inhibiting physiological responses. The absolute potency of particular ligands to suppress binding or affect the physiological response, often differs markedly. However, this could be due to changes in the properties of receptors caused by tissue disruption, or the different conditions in which potencies are determined. Therefore, the validity of the use of ³H-DHEC or other reversible ligands to label functionally relevant receptors must be tested in experiments where the above complications can be reduced or eliminated. We report here the use of an irreversible α -adrenoreceptor antagonist which provided a way to bridge the gap between physiological experiments measuring mechanical responses of an intact tissue and binding assays using membrane fragments. α -Receptors mediating contractions of isolated rabbit uterine strips were irreversibly blocked by phenoxybenzamine (POB), and ³H-DHEC binding was then measured in membranes prepared from these POB-treated and from untreated control strips. The results obtained dissociate phentolamine-suppressible ³H-DHEC binding and α -receptors mediating uterine contraction, but indicate that a fraction of the binding with low capacity and high affinity for DHEC, may represent true postsynaptic α -receptors.

Myometrial strips were prepared from uteri of immature New Zealand rabbits and were mounted in tissue baths containing de Jalon solution at 31 °C. Concentration-response curves for noradrenaline (NA) induced isometric contractions were determined before and after a 20-min exposure to various concentrations of POB. A crude membrane fraction was then prepared from the control and POB-treated strips to test the *in vitro* binding of ³H-DHEC.

POB irreversibly blocked the NA-induced contractions of uterine strips. As little as 10⁻¹⁰ M POB produced significant inhibition, and complete block was achieved by 3 × 10⁻⁸ M (Fig. 1). Spare receptors were not evident in the rabbit uterus, as the threshold blocking concentration of POB (10⁻¹⁰ M) significantly reduced the maximal response to NA. The high blocking potency of POB and the absence of spare α -receptors have also been observed in other smooth muscles^{11,12}. POB treatment of the intact strips significantly reduced the specific binding of ³H-DHEC (8 nM) as compared with the binding to

Fig. 1 Effects of phenoxybenzamine (POB) on noradrenaline(NA)-induced uterine contractions and on the specific binding of ^3H -DHEC to uterine membrane fragments. Suppression of the maximal contractile response to NA (■); suppression of specific ^3H -DHEC binding after incubation of POB with intact strips (△) or with membrane preparations (▲). Points and bars represent mean $\pm$ s.e. of triplicate determinations in 4–9 experiments. Uteri were removed, and the two horns separated and longitudinally halved. The strips were trimmed of fat and endometrial tissue, and suspended in tissue baths containing de Jalon solution (in mM: NaCl, 154; KCl, 5.6; CaCl₂, 0.3; NaHCO₃, 6.0; glucose, 2.8; pH 7.4) at 31 °C, gassed with 5% CO₂ in O₂. Isometric contractions were recorded by force-displacement transducers (Grass FT03C) and a polygraph. Contractions were induced by single graded doses of (–)noradrenaline, after which all but one strip were exposed to various concentrations of POB for 20 min. After a 1-h washout period NA concentration–response curves were redetermined and corrected for any spontaneous change as tested in the strip not exposed to POB. After thorough washing with de Jalon solution for 90 min, the strips were dissected and a crude membrane fraction was prepared³. The strips were minced and homogenised in ice-cold buffer (0.25 M sucrose, 1 mM MgCl₂, 5 mM Tris HCl, pH 7.4, at 25 °C) with a Brinkman polytron (4 $\times$ 6s bursts). The homogenate was filtered through gauze and centrifuged twice at 500g for 10 min at 4 °C. The pellet was discarded and the supernatant centrifuged at 27,000 g (10 min, 4 °C) and washed twice with incubation buffer (10 mM MgCl₂, 50 mM Tris HCl, pH 7.5 at 25 °C), in which the final pellet was resuspended at 3–4 mg ml^{–1} protein. The same method was used to obtain membranes from freshly removed uteri. Aliquots of such membrane preparations were incubated with POB at 25 °C for 12 min (ref. 3). The suspension was then diluted with 50 vol incubation buffer and centrifuged and washed twice at 27,000 g. A control aliquot was treated the same way except for the addition of POB. The binding of ^3H -DHEC (NEN, 27.3 Ci mmol^{–1}) to uterine membranes was assayed by a rapid filtration method³. Incubations were in polyallomer tubes at 25 °C for 15 min and binding was terminated by adding 2 ml of incubation buffer to the 150- μ l aliquots containing 8 nM ^3H -DHEC, and immediate filtering through Whatman GF/C glass fibre filters. The filters were washed for 15 s with 20 ml of the same solution at 25 °C. Specific binding is the difference between binding in the absence and presence of 10 μ M phentolamine, and it was 30–50% of total binding. Control membrane preparations bound 90.6 ± 10.5 fmol ^3H -DHEC per mg protein.



membranes prepared from control strips not exposed to POB. However, the concentration of POB needed to suppress ^3H -DHEC binding was approximately 50 times higher than that required to produce equivalent block of the mechanical response to NA (Fig. 1), and even 10^{-6} M POB did not completely suppress binding. As this discrepancy may have been due to diffusion barriers that limited the access of POB to receptors inside the intact tissue, in a group of experiments POB was incubated with uterine membranes rather than with intact strips. Preparation of the membranes from the freshly removed uteri, incubation with various concentrations of POB and the subsequent assay of ^3H -DHEC binding were as described by Williams and Lefkowitz⁴. Suppression of ^3H -DHEC binding by POB in these preparations was practically identical to the suppression observed after exposure of intact strips to POB (Fig. 1). The 40% decrease in ^3H -DHEC binding by 10^{-7} M POB is comparable to the 45% suppression observed in similar experiments by Williams and Lefkowitz⁴. These observations eliminate the possibility that the dissociation of the effects of POB on the physiological response and on ligand binding could have been due to diffusion barriers in the intact tissue or to breakdown of POB in the incubation medium⁴.

Differences between the results of binding studies and experiments with functioning tissues could also be related to the fact that the ionic composition and temperature of the media used are usually very different. As ^3H -DHEC binding in the present experiments and in earlier studies^{2–5} was tested in Tris buffer at 25 °C, we repeated the last group of experiments using de Jalon solution at 31 °C for the final resuspension of membranes before their incubation with POB and the assay of ^3H -DHEC binding. One-half of each membrane preparation was exposed for 20 min to 3×10^{-8} M POB, the lowest concentration that produced complete block of contractions in strips, and binding of various concentrations of ^3H -DHEC was then tested both in the control and in the POB-treated sample (Fig. 2). POB did not significantly alter specific ^3H -DHEC binding at higher concentrations of the labelled ligand, thus confirming dissociation of these binding sites from α -receptors mediating smooth muscle contraction. However, the same concentration of POB almost completely eliminated specific binding at low concentrations of ^3H -DHEC (≤ 3 nM), and there seems to be a break in the control binding curve at the same ^3H -DHEC concentration. These latter findings suggest that the low-capacity, high-affinity component of DHEC binding (Fig. 2) may

represent true smooth muscle α -receptors with a high sensitivity to blockade by POB.

This possibility was further tested in a 'receptor protection' experiment. Three strips obtained from the same uterus were mounted in tissue baths. After determination of NA concentration–response curves, one strip was exposed for 20 min to 3×10^{-8} M POB alone, the second was exposed to 3×10^{-8} M POB in the presence of 10^{-5} M phentolamine, and the third remained untreated as control. Retesting the effect of NA after repeated washing of the strips for 1 h showed no significant change in the control, complete block in the POB-treated strip and a reduction of the maximal NA contraction from 2.15 to 1.05 g in the strip exposed to both POB and phentolamine (50% protection). The specific binding of ^3H -DHEC (2 nM) in membranes from the same three strips was 40.2, 0 and 22.1 fmol per mg protein, respectively, indicating 55% protection in the last preparation. In two other experiments, methoxamine-induced contractions of strips and suppression of ^3H -DHEC (2 nM) binding in membranes were tested in preparations obtained from the same uterus. Methoxamine was used as agonist, because it is not a substrate for monoamine oxidase, catechol-*O*-methyl transferase or neuronal uptake¹³ and, in the absence of spare α -receptors in the uterus, its EC₅₀ should be similar to its K_d. The observed mean EC₅₀ of 1.5×10^{-6} M for contraction and K_d of 5×10^{-7} M for binding are in good agreement.

The good correlation between irreversible block of the physiological response and suppression of ^3H -DHEC binding by POB, the similar degree of protection against both effects by phentolamine, and the similar absolute potency of methoxamine for contraction and binding suggest that the high-affinity component of ^3H -DHEC binding does, in fact, represent functional α -receptors in smooth muscle. For the first time, ligand binding and receptor-mediated mechanical responses were determined in the same preparation and could be directly compared. The second, high-capacity, binding component does not represent α -receptors mediating uterine contraction and the present results provide no information about the possible physiological role of these more numerous sites. They may be non-functional 'silent receptors' (ref. 14) for catecholamines, which may have affinities for various adrenergic ligands that are similar but not identical to the affinity of true receptors. Alternatively, the second binding component may be related to release-modulating α -receptors. POB is considerably more

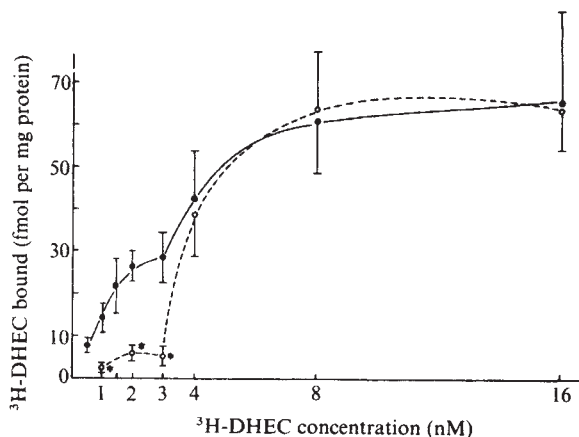


Fig. 2 The effect of phenoxybenzamine on the specific binding of various concentrations of ^3H -DHEC to uterine membranes. Membranes were prepared from freshly removed uteri as described in the legend to Fig. 1, except that final resuspension of membranes before incubation with POB (3×10^{-8} M, 20 min, 31°C) and the binding assay (15 min, 31°C) was in de Jalon solution. Points are means $\pm$ s.e. of triplicate determinations from 4–7 different uteri. POB-treated ($\circ$) and control ($\bullet$) values are matched and membrane material from any one uterus was sufficient to test binding at 4–6 concentrations of ^3H -DHEC. In individual preparations, where at least four points were obtained at or below 3 nM ^3H -DHEC, Scatchard plots of such control values gave a mean K_d of 1.5 ± 0.4 nM and a b_{max} of $36.6 \pm \text{fmol per mg protein}$. In membrane preparations where most points tested were above 3 nM, the K_d of ^3H -DHEC binding was 5.5 ± 1.7 nM and the b_{max} was 97.3 ± 18.8 fmol per mg protein. (1, 2, 4, 8, 16 nM.)

* Indicates significant difference ($P < 0.005$) from the corresponding control value.

potent in blocking post- than presynaptic α -receptor responses¹², and the unexplained high potency of clonidine (K_d 0.23 μM) in suppressing the binding of 8 nM ^3H -DHEC in an earlier study³ is also compatible with the involvement of presynaptic receptors. However, more direct evidence is needed to decide this issue.

The present results should caution against prematurely equating ligand binding sites characterised *in vitro* with receptors subserving a well defined physiological function *in vivo*. ^3H -DHEC binding described in earlier studies in uterine²⁻⁴ or vascular smooth muscle⁵ differed from the high-affinity binding component in the present experiments: the K_d of ^3H -DHEC was 8–10 nM, and only high concentrations of POB suppressed specific binding⁴. This indicates that the sites involved were not smooth muscle α -receptors mediating contraction. Changes in the density of such ^3H -DHEC binding sites in oestrogen- and progesterone-treated rabbit uterus¹⁵ have been suggested to contribute to or explain the reciprocal change in α - and β -receptor-mediated mechanical responses of such preparations. As the binding sites detected are unrelated to smooth muscle α -receptors, the physiological significance of changes in their density remains to be established.

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Substance P inhibits nicotinic activation of chromaffin cells

THERE is much interest in the possibility that substance P acts as a neurotransmitter and/or a neuromodulator at certain sites in the central and peripheral nervous system (for reviews see refs 1, 2). Although both excitatory³⁻⁸ and inhibitory^{6,9-11} actions have been observed *in vivo* and *in vitro*, the cellular mechanism of action of substance P has not been defined. Recent electrophysiological studies in the cat⁹⁻¹¹ have provided evidence that substance P selectively depresses the nicotinic excitation of Renshaw cells by microiontophoretic application of acetylcholine (ACh). We show here that substance P inhibits the ACh-induced nicotinic response of adrenal chromaffin cells in culture to release ^3H -noradrenaline.

Intact and functional chromaffin cells were isolated from bovine adrenal glands by retrograde perfusion of the dissected medullae with collagenase (type 1, Sigma) in a Locke's buffer (pH 7.0) and purified by density gradient centrifugation on

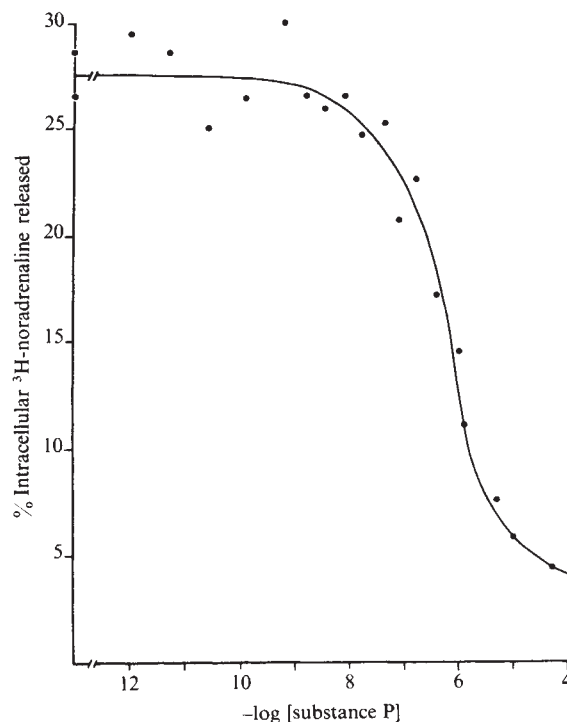


Fig. 1 Inhibition by substance P (10^{-4} – 10^{-12} M; Beckman) of the release of ^3H -noradrenaline from cultured bovine adrenal medullary cells evoked by ACh (5×10^{-5} M). The percentage of the total cellular ^3H -noradrenaline released by 5×10^{-5} M ACh in the absence of substance P is shown on the ordinate. The results of a typical experiment are shown, where each point represents the release from a single, separate culture dish.

Table 1 Effect of substance P (SP) and Ca^{2+} on the release of ^3H -noradrenaline from cultured adrenal chromaffin cells

Secretagogue	Addition	% Intracellular ^3H -noradrenaline released mean $\pm$ s.d. (n)
5×10^{-5} M ACh	2.2 mM Ca^{2+}	20.9 $\pm$ 3.1 (47)
	5×10^{-5} M SP + 2.2 mM Ca^{2+}	3.9 $\pm$ 1.3 (5)
	No Ca^{2+}	1.8 $\pm$ 0.4 (3)
	1 mM EGTA	0.9 $\pm$ 0.1 (3)
5×10^{-6} M nicotine	2.2 mM Ca^{2+}	16.8 $\pm$ 2.4 (5)
	5×10^{-5} M SP + 2.2 mM Ca^{2+}	2.1 $\pm$ 0.4 (3)
56 mM K^+	2.2 mM Ca^{2+}	15.3 $\pm$ 1.5 (7)
	5×10^{-5} M SP + 2.2 mM Ca^{2+}	15.1 $\pm$ 0.9 (2)
	No Ca^{2+}	2.3 $\pm$ 0.2 (3)

Percoll as described previously¹²⁻¹⁴. The purified cells were plated on collagen-coated plastic dishes (Corning 35 mm, 10^6 cells per plate) in Dulbecco's modified Eagle's medium (DMEM; Gibco), pH 7.2, in conditions shown to favour sympathetic neurite outgrowth in culture¹⁵. The cells attached firmly within 3 h, and by 8 h had extended processes with growth cones which eventually made contacts with other cells^{13,16}. The cells and their varicose processes contained high concentrations of catecholamines, as demonstrated by the glyoxylic^{14,17} and formaldehyde-glutaraldehyde^{14,18} fluorescence histochemical methods. By day 4 in culture they exhibited many of the morphological features of noradrenergic neurones in culture.

The pharmacological experiments were carried out on chromaffin cells after 4 and 5 days in culture. The plates were loaded for 2 h at 37°C with ^3H -noradrenaline as described by Greene and Rein¹⁹ for sympathetic nerve cultures. In this technique any unbound amines and metabolites are washed out in a subsequent 2 h period and the washout medium replaced with HEPES-buffered Krebs-Ringer saline (KRH) for 15 min²⁰; however, instead of the 2.6 mM Ca^{2+} concentration used by Greene and Rein we used 2.2 mM Ca^{2+} as our previous work¹² has shown this concentration to effect maximum stimulation of ACh-induced catecholamine release from isolated adrenal medullary cells. After this adaptation period, the plates were incubated in KRH (5 min); KRH containing nicotinic and muscarinic agonists and/or antagonists (5 min); and KRH alone (5 min). Substance P, when tested, was present during the first two 5-min incubation periods. The radioactivity in each of the three incubation media and that remaining in the cells was determined as described by Greene and Rein¹⁹ using a Nuclear Chicago Isocap 300 scintillation counter, with external standards ratio. The results are expressed as the amount of ^3H -noradrenaline released in the 5-min stimulation sample expressed as a percentage of the total radioactivity originally present in the cells.

Pharmacological studies with nicotinic (nicotine, ACh) and muscarinic (methacholine) agonists and their antagonists (mecamylamine HCl; hexamethonium and atropine, respectively) showed that the adrenal chromaffin cells contained only nicotinic receptors. Methacholine (10^{-10} – 10^{-4} M), a muscarinic agonist, did not stimulate the cells to release ^3H -noradrenaline, and the ACh-induced release of ^3H -noradrenaline (ED_{50} 5×10^{-5} M) was not inhibited by low concentrations (10^{-5} – 10^{-10} M) of atropine. By contrast, substance P produced a dose-dependent inhibition of ACh (5×10^{-5} M) stimulated ^3H -noradrenaline release (Fig. 1) in the range 10^{-8} – 5×10^{-5} M, with an ID_{50} of approximately 5×10^{-7} M.

A similar inhibition of noradrenaline release by substance P was obtained when nicotine (5×10^{-6} M) was used as the agonist, but not when K^+ (56 mM) was used to depolarise the cells (Table 1). Omission of Ca^{2+} reduced the release induced by 5×10^{-5} M ACh and by 56 mM K^+ to 1.8% and 2.3%, respectively, of the total intracellular ^3H -noradrenaline, and addition of EDTA (1 mM) or EGTA (1 mM) reduced it to the basal

release level of 0.9% (Table 1). Substance P (5×10^{-5} – 10^{-10} M) by itself did not have a significant effect on the basal release rate of ^3H -noradrenaline from these cells in the presence of 2.2 mM Ca^{2+} .

As pharmacological studies have shown that isolated chromaffin cells^{12,21,22} contain only nicotinic receptors, our present results provide the first direct evidence at a cellular level that substance P acts as an inhibitory modulator of the nicotinic ACh response. Taken with the earlier microiontophoretic studies *in vivo*⁹⁻¹¹, our results support a role for substance P as an inhibitory neuromodulator at nicotinic receptor sites in the nervous system²³.

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Efficient induction of immediate tolerance to contact sensitivity by hapten-modified spleen cells requires Ia⁺ cells compatible with recipient

IMMUNOLOGICAL distinction between 'self' and 'non-self' is important in maintaining normal homeostasis: a breakdown in this regulation probably results in auto-destructive processes. Recent developments in cellular immunology have shown that the induction of an immune response (sensitisation) involves the recognition of antigen in combination with products of the major histocompatibility complex (MHC)^{1,2}. In this context, it is pertinent to ask whether the induction and maintenance of specific immunological unresponsiveness (tolerance) to foreign antigens is also under genetic control. Contact sensitivity and tolerance to simple chemical compounds (haptens) in mice has provided insight into the complex cellular interactions involved in the unresponsive state³⁻⁵. Parenteral injection of the reactive

Table 1 Efficient induction of immediate tolerance to contact sensitivity of DNFB-modified spleen cells requires Ia-bearing cells

Treatment of DNP-SC tolerogen	DNFB sensitisation	DNFB challenge	Ear swelling (inches $\times 10^{-4}$) $\pm$ s.e.m.	% Tolerance
—	+	+	79.2 $\pm$ 3.7	0
NMS + C	+	+	25.8 $\pm$ 3.3*	71
Anti-Ia + C	+	+	59.5 $\pm$ 4.8*	26
—	—	+	3.6 $\pm$ 1.5	—

CBA/J erythrocyte-free spleen cell suspensions were prepared in Hank's balanced salt solution (HBSS). The cells were resuspended to 10^8 cells per ml in HBSS and either NMS or anti-Ia (A.TH into A.TL) antisera added at a concentration of 0.1 ml per 10^6 cells followed by incubation on ice for 60 min. Rabbit complement was then added and the suspension incubated at 37 °C for 30 min. Dead cells were removed by Ficoll-Isopaque gradient centrifugation⁹. Living cells were collected, labelled with DNFB as previously described⁴, and 3×10^6 DNP-SC injected on day 1 intravenously into age-matched CBA/J recipients. The recipient animals were sensitised with either 0.5% DNFB in acetone: olive oil (4:1 v/v) or acetone: olive oil (negative control) on days 0 and 1. On day 5, the recipients were ear challenged with 0.2% DNFB and swelling measured 24 h later. The values represent the mean 24-h ear swelling (4 members per group) $\pm$ s.e.m. The amount of tolerance obtained was calculated by comparing the response of each experimental group to that of positive (sensitised and ear challenged) and negative (ear challenged only) control groups: % Tolerance = [(positive - experimental)/(positive - negative)] $\times 100$ %.

* $P < 0.001$.

haptens 1-fluoro-2,4-dinitrobenzene (DNFB) or its sulphonate (DNBS) before epicutaneous sensitisation with DNFB results in unresponsiveness^{3,4}. This process seems to be mediated by at least two mechanisms, clonal inhibition and induction of suppressor T cells (T_s)⁶, both of which can be induced by either DNBS or DNFB coupled *in vitro* to red blood cells (DNP-RBC). The maximum tolerant state then develops 4–7 d after injection. However, another tolerogen, DNFB coupled *in vitro* to spleen cells (DNP-SC), produces maximum unresponsiveness immediately. This rapid induction of unresponsiveness was shown to be independent of T_s and thus was referred to as clonal inhibition⁶. The dichotomy between syngeneic DNP-SC and DNP-RBC in their ability to tolerise immediately was first thought to be due to either different homing characteristics or cell surface components⁴. With respect to the latter, an intriguing difference between the two tolerogens is the lack of I-region associated (Ia) antigens (of the MHC) on the surface of red blood cells^{7,8}. We have investigated this difference by tolerising mice with DNP-SC depleted of Ia⁺ cells or with DNP-SC from donors which are I-region incompatible with respect to the recipient animal. The results reported here indicate that the induction of immediate tolerance requires I-region compatibility between the donor of the DNP-SC tolerogen and the recipient.

Mice were tolerised with DNP-SC pretreated with either normal mouse serum (NMS) or anti-Ia antiserum plus rabbit complement. After the serum treatment and before labelling

with DNFB, dead cells were removed by differential centrifugation in Ficoll-Isopaque gradients⁹. One day after tolerisation the recipient mice plus appropriate controls were sensitised by two paintings with DNFB. All animals were ear challenged 5 d later and ear swelling measured 24 h later. The results are shown in Table 1. DNP-SC treated with NMS induced 71% tolerance. In contrast, DNP-SC which had been depleted of Ia⁺ cells induced only 26% tolerance, which is equivalent to the amount of unresponsiveness induced by another Ia-negative population of cells, DNP-RBC⁴. This decrease in tolerogenicity of DNP-SC depleted of Ia⁺ cells indicated that Ia⁺ cells are required to produce efficient immediate tolerance.

Further experiments were then done using the proper congenic strains to ascertain whether or not the Ia antigens of the tolerogen had to be identical with the recipient. For this analysis, it is necessary to keep K and D ends of the MHC compatible between the two so as to minimise any allogeneic effects. As seen in Table 2, when the tolerogen was incompatible with the recipient at the I region (DNP-A.TL into A.TH), there was no immediate induction of tolerance (0%) as compared to a syngeneic tolerogen (DNP-A.TH into A.TH), which produced immediate tolerance (53%). These results indicate that if the tolerogen and recipient share identical K and D regions, then I-region compatibility is required to induce efficient immediate tolerance.

The ability to induce unresponsiveness rapidly was also shown in the picryl chloride (PiCl) system to depend on the cell population which was haptenated with picryl sulphonic acid (PSA) *in vitro*⁵. Whereas hapten-coupled spleen and lymph node cells were highly efficient at inhibiting contact sensitivity to PiCl, PSA coupled to erythrocytes and thymocytes were ineffective. As the latter populations are thought to be deficient in Ia antigens^{7,8}, these results support the idea that induction of immediate tolerance requires hapten on Ia-compatible cells.

It must be pointed out, however, that the requirement for Ia-bearing cells is not seen when the sensitisation regimen is delayed. Miller and H.N.C.⁴ could not detect any differences between several haptenated cell populations (spleen, lymph node, peritoneal exudate cells and thymus) in their ability to induce tolerance to DNFB if the animals were tolerised 7 d before sensitisation. A possible explanation for this apparent inconsistency would be that host reprocessing has occurred during the longer time interval, such that the tolerogen is now expressed on the proper MHC background.

Host processing might also explain the observation that completely allogeneic DNFB-labelled cells (that is, K, D and I region incompatible) are as efficient as a syngeneic tolerogen in their ability to induce tolerance¹⁰. It is possible that alloreactivity might accelerate or somehow alter normal host processing, thereby allowing re-expression of the tolerogen on a self-MHC background. Therefore, I-region compatibility between the DNP-SC donor and the recipient is not required when K and D regions are different.

It is unlikely that the tolerant state induced here by DNP-SC is due solely to the production of a significant number of T_s because of the rapidity with which the unresponsiveness develops^{4,6}. Further evidence for the delay in the development

Table 2 Efficient induction of immediate tolerance to contact sensitivity by DNFB-modified spleen cells requires I-region compatibility between the tolerogen and recipient

Donor of DNP-SC tolerogen (KID)	DNFB sensitisation	DNFB challenge	24-h ear swelling (inches $\times 10^{-4}$) $\pm$ s.e.m.	% Tolerance	MHC compatibility between tolerogen and recipient
—	+	+	60.0 $\pm$ 3.8	0	—
A.TH (ssd)	+	+	35.2 $\pm$ 2.0*	53	KID
A.TL (skd)	+	+	60.7 $\pm$ 2.9*	0	KD
—	—	+	13.3 $\pm$ 3.1	—	—

Donor spleen cells were prepared and labelled as described in Table 1. 3×10^6 DNP-SC were injected intravenously into A.TH recipients. The recipients were then sensitised and challenged as in Table 1.

* $P < 0.001$.

of T_s is the fact that immune lymph node cells are not inhibited when transferred into animals tolerised 1 d previously with DNP-SC⁶. Also, cyclophosphamide pretreatment (which eliminates T_s precursors) does not alter the kinetics of immediate tolerance induction⁶. Therefore, we believe that DNP-SC is immediately inducing clonal inhibition. The tolerogen may act by blocking or inhibiting the T-cell receptors, thus preventing expansion of the specific clones after antigenic stimulation.

The exact role of Ia-bearing cells in the induction of immediate tolerance is unclear. However, one interpretation is that some T-cell clones which are required for the development of contact sensitivity may recognise DNFB in association with I-region gene products. Other investigators have reported that T cells recognise antigen plus surface MHC structures in both the induction and/or elicitation of immune responses, including delayed hypersensitivity¹¹, T-cell-B-cell collaboration¹², cytotoxic T-cell killing of virus-infected¹³ or hapten-modified target cells¹⁴, and T-cell proliferation *in vitro*^{1,2}.

The above mentioned studies indicate that the presentation of antigen in association with macrophage Ia determinants activates the T cell. In contrast, our results suggest that perhaps antigen, in association with an Ia-bearing cell, is capable of tolerising T cells. Thus, the decision for either tolerance or immunity depends not only on the nature of the ligands presented (antigen with Ia) but also on either their pathway of presentation (perhaps the tolerogenic action of DNP with Ia⁺ cells is due to the fact that it is not presented on macrophages) or their delivery with or without a second signal. This second signal would be required to activate the T cell; in its absence, tolerance results¹⁵.

It should be noted, however, that the relationship between hapten and Ia determinants has not been completely resolved. The hapten PSA has been shown to derivitise murine H-2 antigens *in vitro*¹⁶ but not the Ia-like structure in strain 13 guinea pigs¹⁷. Therefore, although both the hapten and Ia are required in immediate tolerance induction, the hapten may not be covalently attached to Ia but rather associated with some other structure on the Ia⁺ cells. In this case, the interaction is between the hapten-bearing cell and the target cell. Further experiments are underway to determine the exact role of Ia in the induction of immediate tolerance.

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Serum amyloid P-component is an acute-phase reactant in the mouse

THE acute-phase reaction in mammals is characterised by an increase in the concentration of certain plasma proteins which starts within hours or days of most forms of acute tissue damage or inflammation, and persists with chronic inflammation and malignant neoplasia¹⁻⁴. Among acute-phase reactants are protease inhibitors, coagulation factors, complement components and transport proteins as well as proteins without known *in vivo* roles, such as the classical reactant, C-reactive protein (CRP)¹⁻⁴. The pathophysiological significance of the acute-phase reaction is not known, but it is a usual accompaniment of tissue damage, and it is of clinical value in the assessment of disease activity⁴. We now report that in the mouse, serum amyloid P-component (protein SAP)⁵, which is a stable plasma protein in man⁶, is a major acute-phase reactant. In addition, our observations facilitate investigation of the function of protein SAP and of the pathogenesis of amyloidosis, which is a serious disease in man.

Mouse SAP was isolated in different experiments from normal serum, acute phase serum following croton oil injection and ascitic fluid induced either by repeated intraperitoneal injection of Freund's complete adjuvant or by growth of the lymphocytoma McPc 1748 (ref. 7). Mouse CRP was isolated from the same acute-phase sera and ascitic fluids. The isolation procedures were those previously described for the human counterparts^{8,9} and are detailed specifically for the murine proteins elsewhere¹⁰. The SAP from the different sources was indistinguishable. Its behaviour on gel filtration was comparable to that of human SAP¹⁰, and it was homogeneous in gradient

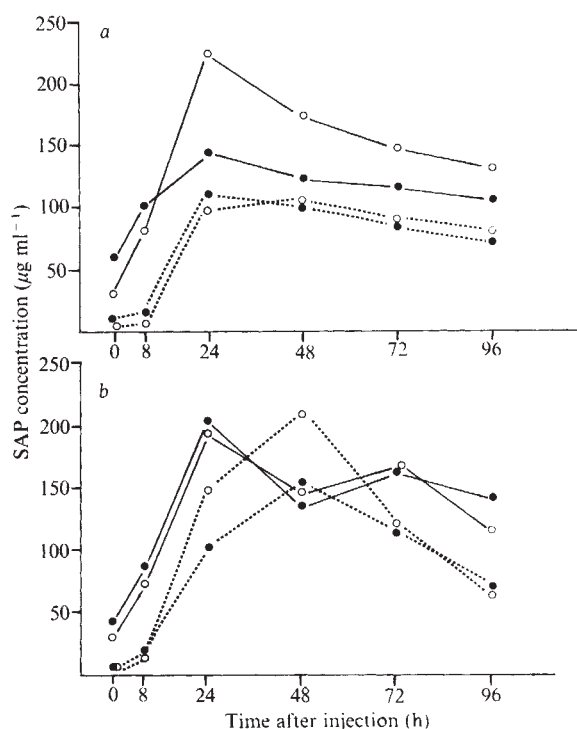


Fig. 1 Effect of acute inflammation on murine serum levels of protein SAP. *a*, Subcutaneous injection of 0.2 ml of a 1% croton oil in liquid paraffin; *b*, intraperitoneal injection of 100 µg of *S. enteritidis* lipopolysaccharide W (Difco). Solid lines, CBA/Lac mice; broken lines, C57BL/Cbi mice; ○, males; ●, females. There were 5 mice in each group and the values shown are the arithmetic means of all animals tested individually in each group. Standard deviations are not plotted but the coefficient of variation was generally <10% except at the peak values when it was 15-25% in all groups.

polyacrylamide gel electrophoresis (PAGE) and reduced SDS-PAGE¹⁰, with a single subunit of molecular weight 25,300 (ref. 11). In the electron microscope mouse SAP was seen as pentameric disks, 8 nm in diameter, with a tendency to interact in pairs face-to-face (E. A. Munn, A. Feinstein and M.B.P., unpublished observations); this closely resembles human SAP⁹. Amino-terminal analysis revealed a blocked N terminal (C. Milstein, A. Feinstein and M.B.P., unpublished observations). These features, and the serum concentrations reported below, distinguish mouse SAP from serum amyloid A (SAA) and other known proteins.

Immunisation with the isolated SAP induced a monospecific rabbit antiserum which was used in an electroimmunoassay¹² of serum SAP calibrated with the pure protein. Murine serum CRP was measured by electroimmunoassay using a monospecific sheep anti-human CRP which cross-reacted with mouse CRP; isolated mouse CRP served as the standard.

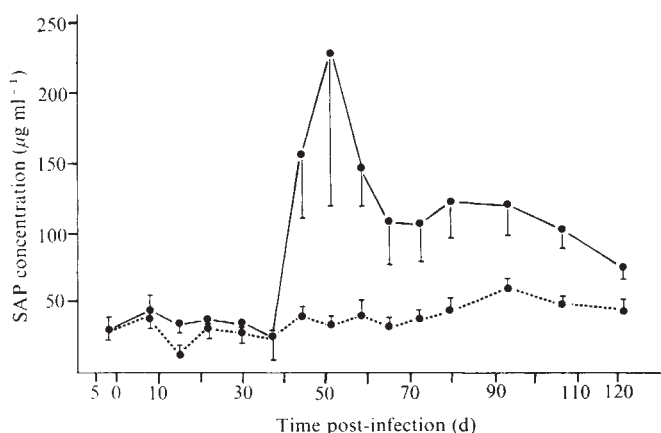


Fig. 2 Effect of *S. mansoni* infection on serum levels of protein SAP in male CBA/Lac mice. Each point represents the arithmetic mean with 1 s.d. of 6 animals in each group. Solid lines, mice infected percutaneously with 20 cercariae¹³ on day 0; broken lines, non-infected age-matched controls.

SAP concentrations rose four- to six-fold in the sera of CBA mice and up to 50-fold in C57BL/Cbi mice within 24–48 h of injection of *Salmonella enteritidis* lipopolysaccharide W (LPS) or croton oil (Fig. 1). On day 32 after the injections, the levels were at pretreatment values in the LPS experiment but were still elevated in the mice given croton oil (100 µg ml⁻¹ in CBA, 10–20 µg ml⁻¹ in C57BL/Cbi mice). Forty days after infection of CBA mice with cercariae of *Schistosoma mansoni*¹³ the serum levels rose sharply and remained elevated (Fig. 2). This coincides with the time of incidence of granulomatous inflammation in the liver. Mice which had received implants of isogenic or xenogeneic tumours also had raised serum SAP concentrations (Fig. 3).

The elevation of murine SAP levels after both acute stimuli and more protracted events, such as parasitic infestation and tumour growth, differs from the behaviour of SAP in man. The normal human serum level of SAP is 30–40 µg ml⁻¹; it does not alter significantly after major surgery and is only slightly increased during chronic active diseases⁶. On the other hand, the level of human CRP in disease ranges up to 600 µg ml⁻¹ (refs 6, 14), whereas, although the mouse CRP concentration rose in the present experiments, it did not exceed 2 µg ml⁻¹. These data agree with the results of a new fluorimetric immunoassay for CRP¹⁵. In man, CRP and SAP share substantial homologies of amino acid sequence, similar subunit composition and comparable appearance in the electron microscope¹⁶, and they both undergo calcium-dependent ligand binding^{8,9}. However, the specific interaction of CRP with pneumococcal C-polysaccharide⁸ and of SAP with agarose⁹ has been used to isolate

them in pure form, and also to identify very similar proteins in the sera of fish, amphibia, birds and non-primate mammals¹¹. These proteins from lower animals share amino acid sequence homologies with their human counterparts and each other (C. Milstein, A. Feinstein and M.B.P., unpublished observations). The acute-phase behaviour of murine SAP suggests that there may also be a functional overlap between CRP and SAP in different species.

The consistent differences in serum level of protein SAP observed in healthy adult CBA and C57BL/Cbi mice before acute-phase stimulation (Fig. 1) were confirmed in animals of the same strains bred and housed in other institutions. Sera from other healthy normal mice showed a consistent pattern of interstrain variation over a 50-fold range (Fig. 4). F₁ hybrids of high (DBA/2) and low SAP (C57BL/6) strains bred in two different institutions had low SAP levels. Outbred nude (athymic) mice and T-deprived CBA/Lac mice¹⁷ had levels comparable with those of intact animals (Fig. 4), suggesting that T-dependent immunocompetence is not required for maintenance of normal levels. The commensal flora are also not essential, as serum SAP concentration was not significantly different in conventional and germ-free CBA/Lac mice (Fig. 4). The observed strain variation suggests a genetic influence on SAP production but it remains to be established whether this is a direct primary effect on the protein synthetic and/or catabolic rates or whether it is secondary to hereditary influences on susceptibility to prevalent acute-phase stimuli such as microbial infection or physical factors.

The normal role of protein SAP *in vivo* is not known, but this protein is a minor constituent of amyloid deposits¹⁸ both in man and in experimental animals. We have recently observed sustained elevation of the serum SAP level during induction of amyloidosis in mice¹⁹, suggesting that it may have pathogenetic significance.

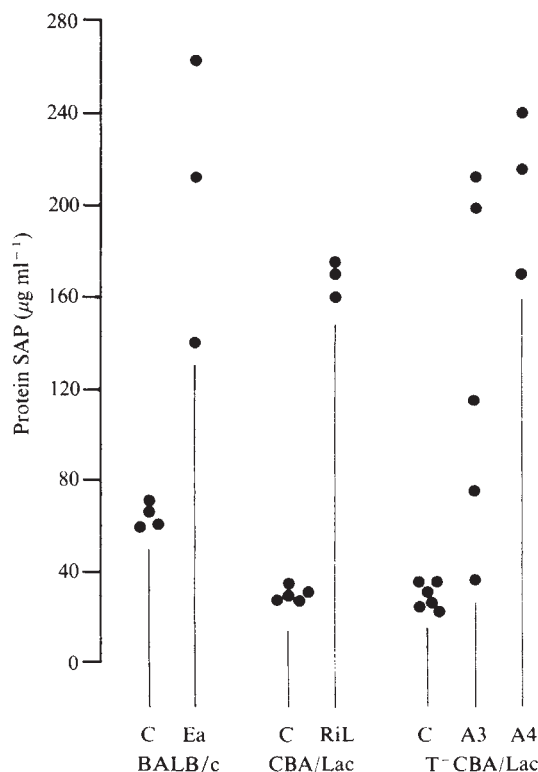


Fig. 3 Effect of tumour implantation on murine serum levels of protein SAP. C, control non-implanted mice; Ea, Ehrlich's ascites tumour, 5 d post-implantation; RiL, radiation-induced leukaemia, 6 d post-implantation; A3, human astrocytoma grade III, 6 d post-implantation; A4, human astrocytoma grade IV, 3 months post-implantation; T⁻CBA/Lac, T-deprived CBA/Lac mice¹⁷.

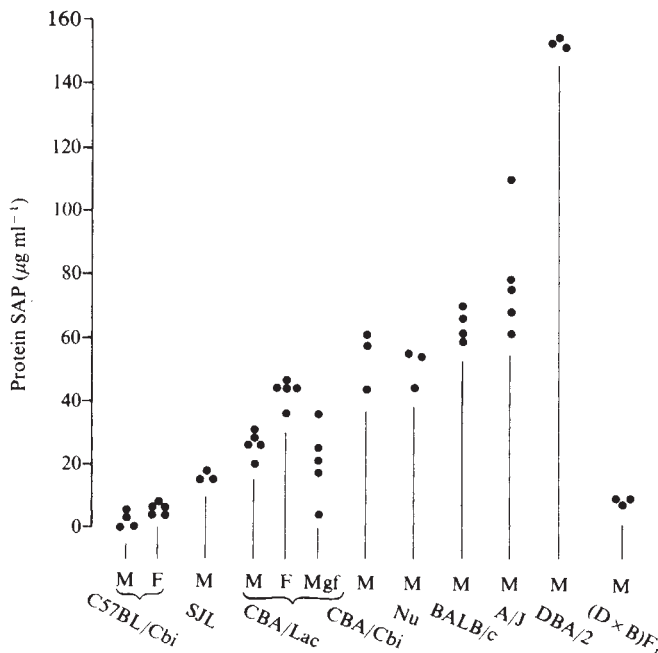


Fig. 4 Serum levels of protein SAP in healthy adult mice of different strains. Each point represents a single estimation in an individual animal. M, males; F, females; Mgf, germ-free males; Nu, outbred nude mice; (D×B)_{F1}, (DBA/2×C57BL/6)_{F1} hybrids.

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T antigen is bound to a host protein in SV40-transformed cells

THE early region of the small DNA tumour virus, simian virus 40 (SV40), is known to code for at least two polypeptides, the t and T antigens ('small t' and 'large T'). Both these polypeptides are expressed in cells transformed by the virus^{1–4}, and the T antigen has been shown to be essential for both the initiation and maintenance of the transformed state^{5–9}. We therefore need to know how this T protein interacts with components of the host cell in order to understand the mechanism of SV40-induced transformation. We report here that the T antigen in a line of SV40-transformed mouse cells forms an oligomeric complex with a specific cell coded protein.

When an extract of the SV40-transformed mouse cell line SVA31E7 is immunoprecipitated by as little as 1 µl of a rabbit antiserum raised against purified T (ref. 10), two polypeptides are clearly specifically immunoprecipitated (Fig. 1, tracks 0, 1). The most slowly migrating species has a molecular weight of 94,000 and is identical to the T antigen precipitated from extracts of SV40 lytically infected monkey cells^{10,11}. The faster migrating species has an approximate MW of 53,000 (53 K) and has not been detected in SV40 lytically infected monkey cells. These same two polypeptides were also specifically immunoprecipitated by other anti-T sera: hamster anti-T sera (raised by inoculating the animals with the SV40-transformed hamster cell line, H65), mouse anti-T sera (raised by repeatedly immunising BALB/c mice with the SV40-transformed mouse cell line, SVT2 (ref. 12)), and anti-U sera, which were raised in monkeys and are specific for the C-terminal end of T (refs 13,14).

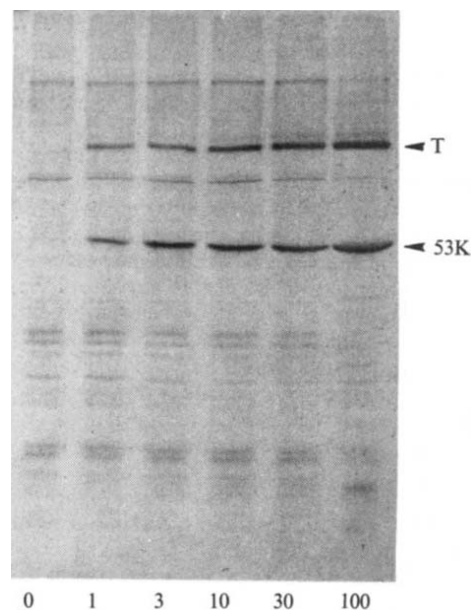


Fig. 1 Quantitation of T and 53K immunoprecipitation by rabbit anti-T serum. An equivalent aliquot of an NP40 cell extract of ³⁵S-methionine-labelled SVA31E7 cells was added to each of 6 tubes followed by normal rabbit serum and rabbit anti-T serum in the following respective amounts: track 0, 30 µl, 0 µl; track 1, 29 µl, 1 µl; track 3, 27 µl, 3 µl; track 10, 20 µl, 10 µl; track 30, 0 µl, 30 µl; track 100, 0 µl, 100 µl. After 3 h incubation at 4 °C, 100 µl of a 10% suspension of fixed *Staphylococcus aureus*²⁰ was added to each tube and following a further 10 min incubation the bacteria were washed 3 times in NET buffer²⁰ and collected by centrifugation. Bound proteins were eluted in 55 µl of sample buffer and 10 µl of each eluate loaded on a 7–20% linear gradient acrylamide gel. The dried gel was autoradiographed for 24 h.

The immunoprecipitation of the 53K protein by the rabbit anti-T serum has two possible explanations, either that the 53K protein shares common antigenic determinants with the gel-purified T antigen used to immunise the rabbit or that the protein is bound in a complex to another species that has such antigenic determinants. The 53K protein could then be specifically immunoprecipitated by the anti-T serum in a 'piggy-back' fashion, in the same way, for example, as antisera to human $\beta 2$ -microglobulin immunoprecipitate HLA antigen¹⁵.

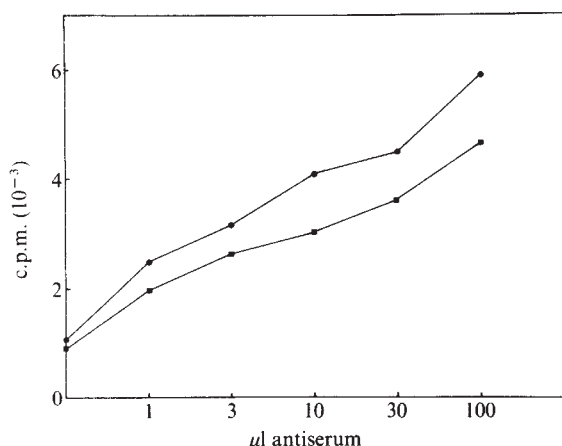


Fig. 2 The strips of dried polyacrylamide gel containing the T antigen (●) and 53K protein (■) from each track were excised, using the autoradiograph as a template, from the gel shown in Fig. 1. Each strip was solubilised²¹ and counted. The c.p.m. present in each band in each track are plotted against the input of rabbit anti-T serum used to immunoprecipitate the cell extract.

To distinguish between these two possibilities we first quantitated the ability of the rabbit anti-T serum to immunoprecipitate the 53K protein, and the T antigen from an extract of SVA31E7 cells. The precipitation ratio for the two proteins (c.p.m. in T/c.p.m. in 53K) remained essentially constant over a 100-fold range of anti-T serum input (Figs 1, 2). Therefore, either the serum has a very similar titre against both species, that is, the 53K protein and T share the majority of the antigenic determinants recognised on T by this antiserum, or the 53K protein is being immunoprecipitated because it is bound to T.

Normal rabbit serum does not precipitate a detectable 53K band from untransformed mouse 3T3 A31 cells. This may be due to one or both of two reasons. Either the cells do not contain the 53K protein or, as there is no T with which it can complex, the 53K protein present is not precipitated by the rabbit antiserum specific for T. A similar experiment to that shown in Figs 1 and 2 with a lower input of cell extract showed that the ratio of T to 53K protein remained the same in serum excess.

It is known that the rabbit anti-T serum, which was raised against T antigen purified by SDS-polyacrylamide gel electrophoresis, recognises a set of determinants on T that are very stable, being resistant to heat, SDS and disulphide bond reduction (ref. 10 and D.P.L. and L.V.C., unpublished). Therefore, we used a direct binding radioimmunoassay to determine whether the isolated 53K protein possessed these antigenic determinants. The results of this assay (Fig. 3) indicate that 53K protein separated from T by SDS-gel electrophoresis is no longer precipitated by the rabbit anti-T serum, whereas the T antigen isolated in the same way is still fully reactive. This shows that the determinants recognised by the rabbit anti-T serum on T are absent from the 53K polypeptide. The immunoprecipitation of the 53K protein from cell extracts by this serum must therefore be due to the fact that it exists in a complex with T. Preliminary gel chromatography results are consistent with this

idea. T and the 53K protein eluted together from Sephacryl S-200 (exclusion limit 0.25×10^6) in the excluded volume (Fig. 4). Sucrose gradient analysis of extracts of transformed cells has shown that the 53K protein and a fraction of the T antigen sediment together at about 14–16S (F. McCormick, personal communication). Co-elution or co-sedimentation clearly do not prove that T and the 53K protein occur in the same complex but are consistent with the complex idea.

We then tested all the other sera which had immunoprecipitated T antigen and the 53K protein from the cell extract to see whether they could bind to the isolated 53K protein and T antigen. The anti-U serum and several different batches of hamster anti-T sera behaved like the rabbit anti-T polypeptide serum in that they efficiently re-immunoprecipitated the gel-purified T antigen but failed to bind the isolated 53K polypeptide. In contrast, the hyperimmune mouse anti-T serum specifically bound both the isolated 53K polypeptide and the T antigen (Fig. 3a, c). The binding of this mouse serum to the gel-purified T antigen from the SVA31E7 cells could be completely inhibited by an unlabelled extract of SV40-infected monkey cells but this treatment did not reduce its titre against

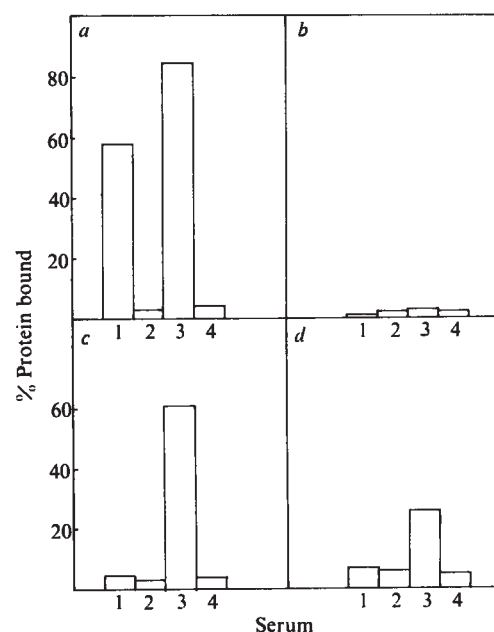


Fig. 3 Serum binding to purified polypeptides. Polypeptides for binding assays were purified from ^{35}S -methionine-labelled cell extracts by immunoprecipitation and SDS-polyacrylamide gel electrophoresis. Cells (PY6 or SVA31E7) were grown in 90-mm dishes in E4 medium with 10% calf serum, and labelled for 3 h with 500 μCi ^{35}S -methionine, then extracted in 0.5% NP40 Tris, pH 8.0, for 30 min on ice¹⁰. To the supernatant of the centrifuged extract, 100 μl of our hamster anti-T serum (for A31E7 cells) or 100 μl of mouse anti-T serum (for PY6 cells) was added. Three hours later 200 μl of *S. aureus* was added and the bacteria washed, collected and eluted as described for Fig. 1. The eluted proteins were separated on 15% polyacrylamide slab gels. The gel fragments containing the polypeptides of interest were located by autoradiography of the dried gel, excised with a scalpel and rehydrated in 2 ml of 0.1 M pH 9 bicarbonate buffer containing 0.1% SDS. Binding assays were carried out as described previously¹⁰ except that 50 μl of *S. aureus* was used in the final step. *a*, Binding to T polypeptide from A31E7 cells by: (1) 10 μl rabbit anti-T serum, (2) 10 μl normal rabbit serum, (3) 10 μl mouse anti-T serum, (4) 10 μl normal mouse serum. *b*, Binding assay control. Binding to a 42K protein from A31E7 cells which is nonspecifically precipitated from cell extracts. Sera as in *a*. *c*, Binding to 53K protein specifically precipitated from A31E7 cells by sera as in *a*. *d*, Binding to 52K protein, specifically precipitated from PY6 cells with mouse anti-T serum, by sera as in *a*. Ordinate is the % of input ^{35}S -labelled protein bound to *S. aureus*. Correction for counter background only.

the 53K protein. Its binding of the 53K protein was, however, inhibited by unlabelled extracts of the SV40-transformed mouse cell lines, SVT2 (the cell line used to immunise the mouse) and SVA31E7 (the cell line from which the purified 53K polypeptide was prepared). Therefore, both the 53K protein and T antigen have unique non-cross-reactive antigenic determinants and the mouse serum contains some antibodies specific for the 53K protein and some specific for T antigen.

This conclusion has been supported by the results of *in vitro* synthesis experiments carried out in collaboration with E.

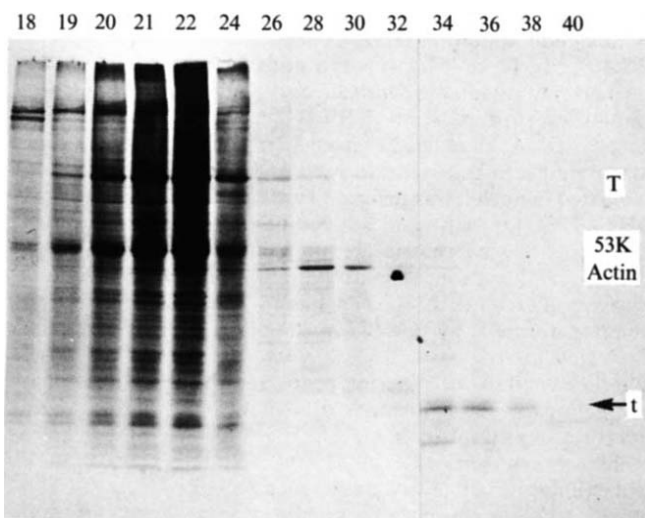


Fig. 4 Gel chromatography of transformed cell extract. Cell extract from ^{35}S -methionine-labelled SVA31E7 cells (10^9 c.p.m.) was applied to a Sephacryl S-200 column ($1.5 \text{ cm} \times 34 \text{ cm}$) and eluted with buffer containing 0.1 M NaCl, 0.01 M Tris, pH 8.0, 1 mM dithiothreitol and 5% glycerol. One-ml fractions were collected and 0.1 ml of selected fractions immunoprecipitated with hamster anti-T serum. The immunoprecipitates were analysed by gel electrophoresis as described for Fig. 1. Each track is labelled with the fraction number, indicating the number of ml from addition of the sample to the column. The void volume of the column was estimated to be 20 ml. Much of the T antigen and 53K protein eluted around this position, whereas t was found around 35 ml and the 42,000 band, thought to be actin, at around 28 ml.

Paucha. When polyadenylated mRNA from SVA31E7 cells was translated in the mouse L-cell protein synthesising system, our hamster anti-T serum immunoprecipitated the T and t polypeptides but not the 53K protein, whereas this same serum could immunoprecipitate both T and the 53K protein from *in vivo* labelled cell extracts. This is not due to a failure to translate the mRNA coding for the 53K protein, as when the same *in vitro* product was immunoprecipitated by the mouse anti-T serum (which contains antibodies to the isolated 53K protein), the 53K protein could then be detected in addition to the T and t polypeptides. This indicates that the T antigen and 53K protein do not assemble to form a complex in the *in vitro* conditions used in the translation reaction.

We have only encountered one other batch of serum able to bind the isolated 53K protein from SVA31E7 cells. This serum was raised in hamsters by inoculation with TSV5 Cl2 hamster transformed cells. Such reactivity explains why P. May *et al.* (in preparation), in contrast to the previous results of E. Paucha (personal communication), have been able to detect the 53K protein in their cell-free translation of polyadenylated mRNA from SV40-transformed mouse cells.

We postulate that the 53K protein is not virally coded because (1) we could not detect it in lytically infected monkey cells, (2) it shares no detectable antigenic determinants with T, but does have its own set of antigenic determinants (immunogenic in syngeneic mice), and (3) the DNA sequence and mRNA map-

ping data indicate that it would be difficult for the early region of SV40 to encode a third polypeptide of 53,000 MW¹⁶⁻¹⁸. If the protein is not coded for by the virus, then it might be detectable in cells transformed by other viruses, by radiation, by chemical carcinogens or even in untransformed cells. In these cases it would not be complexed to T antigen and would therefore be precipitated only by antibodies specific for the 53K protein itself but not by antibodies to T antigen. An initial study supports this idea. When extracts of the polyoma-transformed mouse cell line PY6 were immunoprecipitated with the rabbit anti-T serum, no specific band was detected; immunoprecipitation of the same extract with the mouse anti-T serum (which contains antibodies to the 53K protein) did specifically bring down a band of ~52,000 MW. This cross-reactive protein, when isolated from the gel, was reprecipitated specifically by the mouse antiserum (Fig. 3d). Significantly, it was also specifically bound by the anti-TSV5 Cl2 serum but not by anti-U serum, rabbit anti-T polypeptide serum (Fig. 3d) or our hamster anti-T serum. The 52K protein from PY6 cells was always precipitated to a lesser extent than the 53K protein. For this reason we believe that the two proteins are related but not identical.

It will be necessary to establish further the nature of the 53K protein, but its specific binding to T antigen, and the presence of an antigenically related protein in polyoma-transformed cells imply that it may have a crucial role in the modulation of the transformed state. Thus, it is possible that it acts to neutralise some of the specific functions of T antigen; for example, it could render the cell non-permissive by preventing the T-dependent initiation of viral replication¹⁸ or it may normally act as a regulator of certain cellular functions related to growth control and itself be neutralised by binding to T antigen. It is of prime importance to determine the level of this T antigen binding protein in untransformed cells and normal tissues and to see if it is induced by other carcinogenic agents.

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Note added in proof: The presence of a middle-T antigen species in SV40 transformed rat cells and SV40 transformed mouse cells has recently been reported²²⁻²⁴ and a preliminary characterisation of the protein carried out²⁵.

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Persisting DNA synthesis in SV40-transformed cells in the presence of cycloheximide

INHIBITION of protein synthesis decreases the rate of DNA synthesis in both prokaryotes and eukaryotes. In prokaryotes, DNA synthesis is dependent on protein synthesis only for the initiation of replication¹. In the case of eukaryotes, reduction of DNA synthesis occurs rapidly after the inhibition of protein synthesis^{2,3}, although the mechanism of the tight coupling between protein and DNA synthesis is unclear. The DNA which is synthesised at a reduced rate in these conditions is basically normal except that the elongation step seems to be modified^{2,3}. Newly replicated DNA synthesised in the presence of cycloheximide seems to be associated with less chromatin protein, because chromatin synthesised in these conditions is more susceptible to digestion by DNases^{3,4}. T antigen of simian virus 40 (SV40) is a product of the A gene of the virus⁵ and has been shown to have a role in the establishment and maintenance of transformation⁶⁻⁹. There is evidence that T antigen is required for the initiation of viral DNA replication¹⁰ and the induction of synthesis of cell DNA¹¹⁻¹⁵. It has also been suggested that T antigen initiates DNA synthesis in transformed cells and thereby serves to retain those cells in the transformed state^{10,16-18}. Recently, T antigen was purified almost to homogeneity, and its microinjection into quiescent mouse cells induced DNA synthesis¹⁹. However, the mechanism of action of T antigen in cell DNA synthesis remains to be clarified²⁰. If T antigen acts on cellular DNA synthesis, SV40-transformed cells which contain T antigen may behave differently from other cell lines in their ability to continue DNA synthesis in conditions of protein synthesis inhibition. We report here that DNA synthesis in SV40-transformed cells continued at an almost normal rate while protein synthesis was inhibited by cycloheximide, whereas DNA synthesis in malignant tumour cells without T antigen did not continue in these conditions.

HeLa and SV80, the former derived from human cervical cancer and the latter a human cell line transformed by SV40²¹, were grown in Dulbecco's modified Eagle's medium (Nissui)

supplemented with fetal bovine serum 10% (Gibco) and streptomycin sulphate (200 µg ml⁻¹). Cells were usually cultured for 3-4 d and used when they had reached subconfluence. Evidence of mitosis was observed under the microscope, indicating active growth. Protein and DNA synthesis in the presence of various concentrations of cycloheximide were studied by measurement of the incorporation of radioactive precursors into acid-precipitable material in cells cultured on 13-mm cover slips. As seen in Table 1a, in both cell lines, protein synthesis was reduced by cycloheximide in a concentration-dependent manner. DNA synthesis in HeLa cells was inhibited in the presence of cycloheximide (Table 1b), which was consistent with observations of cycloheximide inhibition of DNA synthesis in eukaryotes¹⁸⁻²⁰. In SV80 cells, however, DNA synthesis was maintained at an almost normal rate in the presence of cycloheximide (Table 1b), although longer treatment of SV80 cells with cycloheximide reduced DNA synthesis gradually. The ³H-thymidine incorporated into cells treated with or without cycloheximide was located in nuclei, was resistant to alkaline hydrolysis (0.3 M KOH at 37 °C for 18 h), and was completely solubilised by acid hydrolysis (10% perchloric acid at 100 °C for 15 min). This resistance of DNA synthesis to inhibition of protein synthesis was seen not only for SV80 cells, but also for such hamster cell lines transformed by SV40 as SVH93, CHLWT15 and CHLA239L1.

We then carried out experiments to determine whether protein fractions such as histones and/or other proteins which would be required for the maintenance of DNA synthesis were supplied at a normal rate in SV80 cells in the presence of cycloheximide. SV80 cells were labelled with ³H-leucine for 3 h in the presence or absence of cycloheximide. Radioactivity and protein content in cytoplasmic and nuclear fractions of the cells and acid-extractable and residual fractions in nuclei were determined. The acid-extractable fraction contained mostly histones, whereas the residual fraction showed no detectable histones when checked by SDS-polyacrylamide gel electrophoresis. Synthesis of proteins from cells treated with cycloheximide was inhibited to approximately 5% of control regardless of the fractions determined (Table 2). The fluorographic pattern of SDS-polyacrylamide gel electropherograms of cytoplasmic and nuclear proteins confirmed none of specific protein retained a normal rate of synthesis with cycloheximide treatment. In the presence of cycloheximide, DNA in newly

Table 1 Incorporation of ³H-leucine (a) or ³H-thymidine (b) into acid-precipitable material in cells treated with or without cycloheximide

Labelling period (h) after cycloheximide	0	Cycloheximide (µg ml ⁻¹)		
		0.5	2.0	5.0
a				
		HeLa (c.p.m. × 10 ⁻² per culture)		
0.5-1.5	24.1 ± 2.2 *	7.48 ± 0.44(31.0)†	3.67 ± 0.12(15.2)	2.97 ± 0.08(12.3)
1.5-2.5	26.7 ± 0.31	7.90 ± 0.70(29.6)	3.98 ± 0.38(14.9)	3.07 ± 0.37(11.5)
2.5-3.5	33.7 ± 2.7	8.17 ± 0.48(24.3)	3.07 ± 0.37(9.1)	3.33 ± 0.06(9.9)
		SV80 (c.p.m. × 10 ⁻² per culture)		
0.5-1.5	86.3 ± 4.6	15.5 ± 0.55(18.0)	9.18 ± 1.5(10.6)	8.60 ± 1.2(10.0)
1.5-2.5	86.7 ± 8.2	15.3 ± 0.44(17.7)	9.64 ± 0.40(11.1)	8.27 ± 0.42(9.5)
2.5-3.5	81.9 ± 4.7	14.8 ± 0.25(18.1)	7.92 ± 0.34(9.7)	7.27 ± 0.60(8.4)
b				
		HeLa (c.p.m. × 10 ⁻³ per culture)		
0.5-1.5	11.1 ± 0.32	3.94 ± 0.47(35.5)	2.22 ± 0.11(20.0)	1.62 ± 0.04(14.6)
1.5-2.5	12.0 ± 0.28	4.41 ± 0.07(36.8)	2.54 ± 0.06(21.2)	1.78 ± 0.52(14.8)
2.5-3.5	11.7 ± 0.18	5.08 ± 0.21(43.4)	2.77 ± 0.16(23.7)	2.19 ± 0.18(18.7)
		SV80 (c.p.m. × 10 ⁻³ per culture)		
0.5-1.5	12.1 ± 0.44	10.9 ± 0.29(90.1)	10.2 ± 2.71(84.3)	11.6 ± 1.12(95.9)
1.5-2.5	11.6 ± 2.2	11.0 ± 1.5(94.8)	9.9 ± 1.39(85.3)	12.3 ± 0.73(106.0)
2.5-3.5	14.0 ± 2.3	10.2 ± 1.2(72.9)	10.4 ± 1.08(74.3)	12.7 ± 0.91(90.7)

Cells were plated on 13-mm cover slips in a multiwell culture tray (Costar) with 1 ml of medium and cultured for 3-4 d until subconfluent. After 0.5, 1.5 or 2.5 h of cycloheximide addition, L-[4,5-³H]leucine (50.6 Ci mmol⁻¹, Amersham) was added at a final concentration of 13 µCi ml⁻¹ and cells were labelled for 1 h. Medium was discarded and the cells were washed twice with cold phosphate-buffered saline, four times with 10% cold trichloroacetic acid, and finally with ethanol. After drying, 10 ml of scintillation cocktail (4 g diphenylloxazol in 1 toluene) was added and radioactivity was counted. b, As in a except that [6-³H]thymidine (26.6 Ci mmol⁻¹, Amersham) was added at a final concentration of 13 µCi ml⁻¹.

* Mean of 3 determinations ± s.e.m.

† % Of control without cycloheximide.

Table 2 Inhibition of protein synthesis by cycloheximide

Fractions	Protein (mg)		Radioactivity (c.p.m. $\times 10^{-4}$)		Specific radioactivity (c.p.m. $\times 10^{-4}$ per mg)	
	Control	Cycloheximide	Control	Cycloheximide	Control	Cycloheximide
Whole cell	6.66	6.58	128.7	4.88	19.33	0.74(3.8)*
Cytoplasm	3.30	3.77	57.4	2.80	17.40	0.74(4.3)
Nuclei	1.50	1.50	47.2	2.03	31.44	1.35(4.3)
Acid extractable	1.28	0.76	10.0	0.30	7.89	0.39(5.0)
Residual	0.28	0.19	19.3	0.56	68.8	2.97(4.3)

SV80 cells were labelled with L-[4,5- ^3H]leucine for 3 h from 0.5 to 3.5 h after addition of $5.0 \mu\text{g ml}^{-1}$ of cycloheximide. Cells were then washed twice with phosphate-buffered saline, homogenised in reticulocyte standard buffer (10 mM Tris-HCl, pH 7.4; 10 mM NaCl; 1.5 mM MgCl_2), and spun down through a 0.88 M sucrose layer to pellet nuclei. Supernatant including sucrose cushion was saved as cytoplasmic fraction. Acid-extractable and residual proteins from nuclei were separated by the 0.25 M H_2SO_4 method²⁷. All fractions were pelleted and washed with 10% cold trichloroacetic acid, except for the acid-extractable fraction which was pelleted by adding 4 volumes of ethanol²⁷. Pellets were dissolved in 0.2 M NaOH. Radioactivity was measured after mixing with scintillation cocktail PCS (Amersham). Protein was assayed by the method of Lowry *et al.*²⁸.

* % Of control without cycloheximide.

synthesised chromatin of both SV80 and HeLa cells was more susceptible to digestion by micrococcal nuclease, as compared with that in control without cycloheximide, suggesting a reduced assembly of proteins into newly synthesised chromatin. These results indicated that DNA synthesis in SV80 cells continued at an almost normal rate under a reduced supply of new proteins in the presence of cycloheximide.

As inhibition of DNA synthesis by cycloheximide was reported to occur very rapidly, within a few minutes^{2,3}, certain proteins with a very short half life were assumed to be required for the maintenance of DNA synthesis, although isolation and characterisation of these proteins have been unsuccessful²². SV80 cells contained high amounts of T antigen among SV40-transformed cell lines²³. T antigen was located almost exclusively in nuclei²⁴ and was bound to double-stranded DNA²⁵ and chromatin²⁶. The fact that T antigen was required for the initiation of each round of viral DNA synthesis⁶ and the fact that the size of replicon units in SV40-transformed cells was smaller than that in normal cells¹⁸ suggested that T antigen may be involved in cell DNA synthesis. These and the following results seem to favour the idea that T antigen may maintain cellular DNA synthesis despite the absence of protein synthesis, directly or indirectly: (1) in all four SV40-transformed cell lines of human and rodent that we have tested, DNA synthesis continued in the presence of cycloheximide; (2) in cells transformed by the *tsA* mutant of SV40 virus, CHLA239L1, which contained temperature-sensitive T antigen, DNA synthesis continued at the permissive temperature, but was inhibited at the non-permissive temperature in the presence of cycloheximide; (3) variation in the resistance of DNA synthesis to inhibition of protein synthesis among SV40-transformed cell lines correlated with the concentration of T antigen in cells. It should be emphasised, however, that these results do not prove a direct action of T antigen on cell DNA synthesis, but may provide us with a new approach for the analysis of the function of T antigen in transformed cells.

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Sodium channels in rabbit cardiac Purkinje fibres

SODIUM channels are essential for normal impulse conduction in the heart and are a major target for the therapeutic action of antiarrhythmic drugs. However, these channels have not been well studied due to the difficulty of measuring cardiac sodium currents under voltage clamp. Previous efforts have not been generally accepted because of doubts about the adequacy of voltage control^{1–8}. The absence of direct information on cardiac sodium channels is unfortunate as evidence exists suggesting that they differ significantly from sodium channels in other excitable membranes. First, blockade of sodium-dependent impulses in heart requires tetrodotoxin (TTX) at 10^{-3} – 10^{-4} times greater concentrations than in nerve or skeletal muscle⁹. Second, the effect of TTX in heart appears to be voltage-dependent¹⁰, unlike its action on sodium currents in other excitable cells^{11,12}. These findings raise the possibility that cardiac sodium channels may have novel structural properties. Further progress depends on the development of reliable measures of sodium current in the heart itself. We report here voltage-clamp recordings of sodium currents in rabbit Purkinje fibres satisfying a number of criteria for adequate voltage control. The experiments indicate the feasibility of characterising sodium channels in an intact mammalian cardiac preparation and open the way for the direct analysis of antiarrhythmic drug action on cardiac conducting tissue.

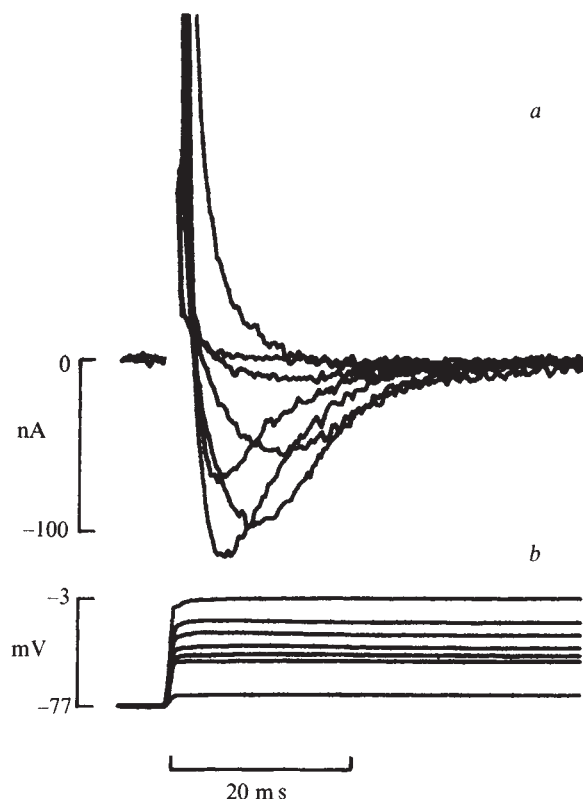


Fig. 1 Sodium currents in the rabbit Purkinje fibre. Membrane currents elicited by depolarisations from a holding potential of -77 mV. Traces are the average of two sweeps (10 kHz filtering) and have been corrected for steady current by computer subtraction. (The size of the steady current is evident in Fig. 2.) Command pulses were exponentially lagged to allow higher clamp gain without ringing and to protect the preparation by limiting the initial surge of capacitive current. Cylindrical surface area, 0.0032 cm^2 ; total capacity, 18 nF . Fibre T46-1, O Ca (5.4 mM Mn), 13°C .

The experiments were carried out in rabbit Purkinje fibre preparations using the two-microelectrode voltage-clamp technique. Johnson *et al.*^{13,14} suggested the choice of the rabbit Purkinje fibres because of its structural advantages over other preparations. It lacks transverse tubules, unlike mammalian ventricular muscle, and it has wide ($1 \mu\text{m}$) intercellular clefts, unlike sheep Purkinje fibres or frog myocardium. The absence of narrow or long extracellular spaces is important in avoiding undesirable external voltage drops. The structural features of the rabbit Purkinje fibre are consistent with its electrical properties. Shortened rabbit Purkinje fibres behave like simple, terminated RC cables, with an extremely low external series resistance to transmembrane current flow^{15,16}. In studying the rabbit Purkinje fibre, we avoided the double sucrose gap method used in earlier studies^{14,17} because of the reported poor viability of the preparations and the problem of extracellular shunt currents¹⁴. We chose instead the two-microelectrode method¹⁸, having found that it allows normal resting and action potentials to be recorded over periods up to several hours long^{15,16}. The main obstacle when studying I_{Na} is longitudinal nonuniformity; this was reduced to an acceptable level by using a low external sodium concentration (15 mM), and short preparations (450 – $700 \mu\text{m}$) to restrict the distance from the current passing electrode to either of the healed-over ends. Reduction of sodium conductance by TTX is less appropriate in these experiments because, as Baer *et al.* have suggested¹⁰, its inhibitory effect is voltage-dependent (T.J.C., C. J. Cohen and R.W.T., unpublished). Temporal resolution of I_{Na} was improved by cooling (13 – 23°C). Interference from other ionic currents was not a serious problem as I_{Na} is much faster and larger than other

Table 1 Voltage-dependent inactivation of sodium current

Expt	Solution	Temperature ($^\circ\text{C}$)	V_{test} (mV)	E_h (mV)	k (mV)
T42-3	5.4 Ca	22	-13	-72	5.45
T44-5	5.4 Ca	19	-24	-66	3.83
T45-1	1.8 Ca + 3.6 Mn	19	-24	-65	3.71
T48-2	5.4 Mn	26	-34	-83	6.64
T48-6	5.4 Mn	23	-35	-83	7.17
mean				-74 ± 4	5.36 ± 0.71
$\pm \text{s.e.m.}$					

Analysis of $h_\infty(E)$ as shown in Fig. 4. Sodium currents were measured at the test potential (V_{test}) indicated. E_h and k were evaluated for each experiment by fitting the data with equation (2).

components such as the slow inward current or delayed potassium current. These currents and twitch contraction were largely inhibited in most experiments by including manganese ion in the external solution. The composition of the external solution was as follows (mM): 15 NaCl , 135 choline-Cl (Mallinckrodt), 4 KCl , 0.5 MgCl_2 , 10 Tris-buffer ($\text{pH } 7.4$), 5 glucose , and either 5.4 CaCl_2 or $1.8 \text{ CaCl}_2 + 3.6 \text{ MnCl}_2$ or 5.4 MnCl_2 . (See Table 1 and Figure legends for concentrations of Ca or Mn.) Records of membrane current and intracellular potential were taken with a PDP 8/e computer after low pass filtering at 10 kHz .

Figure 1 shows sodium currents (a) produced by step depolarisations from a holding potential at -77 mV (b). The traces in a show total membrane current (capacitive plus ionic) with a small component of steady current omitted. The depolarising step to -68 mV evokes an outward capacity transient but no significant time-dependent ionic current. Larger steps to -46 , -41 , -37 and -28 mV produce progressively greater surges of I_{Na} and progressively earlier peak current. The largest inward current is associated with the step to -28 mV . With further depolarisation, the transient current becomes smaller (-19 mV) and eventually reverses (-3 mV).

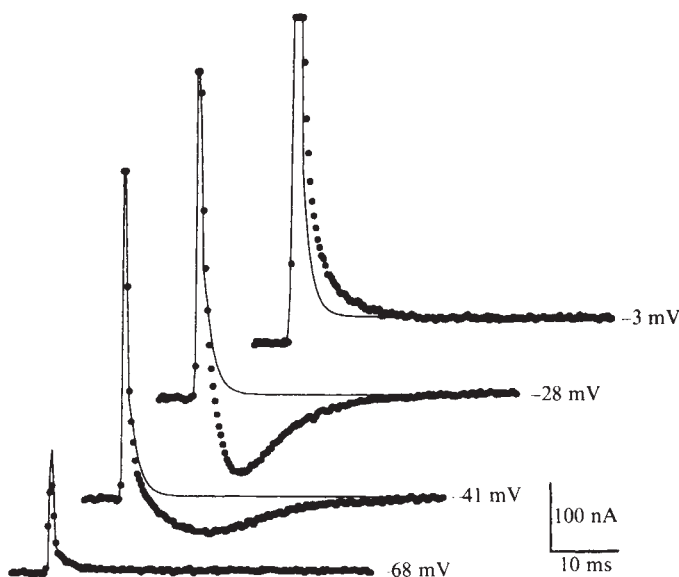


Fig. 2 Procedure for correcting current records for linear capacitative components. The current response to a subthreshold depolarisation to -59 mV was fitted by connecting the first six data points (1.8 ms) and fitting later points by a single exponential ($\tau = 1.3 \text{ ms}$). The resulting function was then scaled in proportion to the size of the voltage step for other depolarisations (smooth curves), and superimposed on the experimentally measured currents (points). The points and the smooth curves both include a component of steady current. The lowest trace (-68 mV) shows that the fit is reasonable for a smaller subthreshold step. The peak of the capacity transient in the upper three traces (-41 , -28 , -3 mV) was off scale and is not resolved. Fibre T46-1.

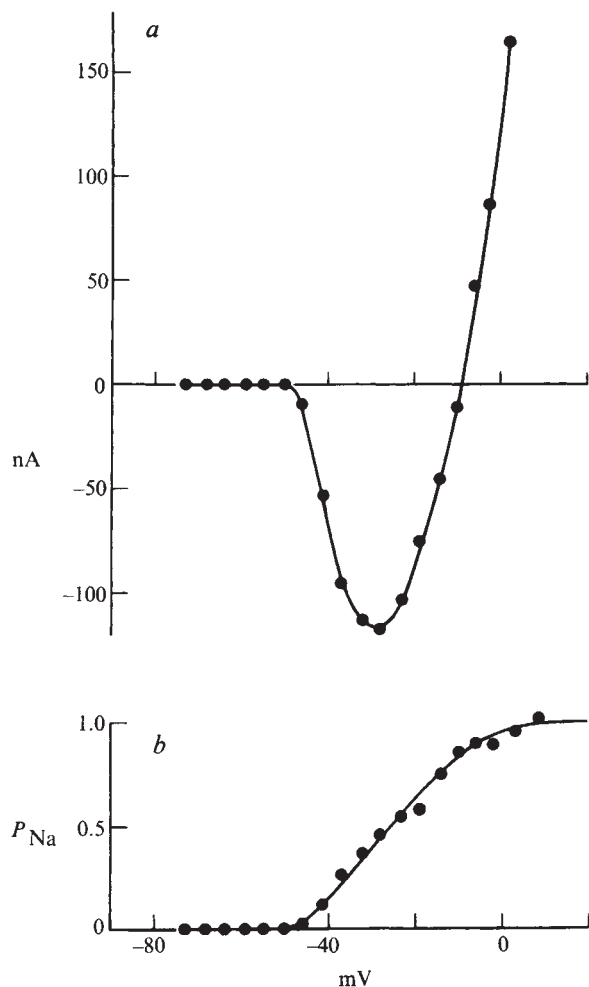


Fig. 3 Voltage dependence of sodium current and sodium permeability in the rabbit Purkinje fibre. *a*, measured currents corrected for capacity and steady current and plotted against membrane potential. Inward values represent peak inward currents. Outward peaks were difficult to resolve accurately because of rapid activation at strong depolarisations. Outward values were therefore measured isochronally at 1.8 ms. *b*, P_{Na} -voltage relationship in the rabbit Purkinje fibre. Sodium permeability calculated from *a* using equation (1) and normalised. When expressed in terms of the external cylindrical surface area of the preparation, maximal P_{Na} is $6.3 \times 10^{-5} \text{ cm s}^{-1}$. This estimate incorporates some resting inactivation at the holding potential, -77 mV. Fibre T46-1.

Sodium current was separated from capacitive current by the procedure shown in Fig. 2. The time course of the capacity transient was obtained from the response to an 18-mV step which was below the threshold for I_{Na} . The smooth curves show this time course, after scaling appropriate to the amplitude of other voltage steps. As expected, the smooth curve provides a good fit to the capacity transient during an even weaker step (record labelled -68 mV). At stronger depolarisations, I_{Na} can be seen as the difference between the smooth curve and the data points. The activation of I_{Na} can be largely separated from the decay of the capacity transient during the step to -41 mV. Temporal resolution is less satisfactory with stronger depolarisations where I_{Na} turns on more quickly. Nevertheless, the difference signal clearly shows outward ionic current at -3 mV.

Peak ionic currents obtained by this analysis are plotted in Fig. 3a. The voltage-dependence of peak I_{Na} is qualitatively similar to that found in other excitable tissues. The reversal of the ionic current takes place near -9 mV. This value is reasonably close to -3 mV, the value calculated from the Goldman-Hodgkin-Katz potential equation^{20,21} with P_{Na}/P_K set at 12 (ref. 22) and Na and K activities estimated by ion-sensitive microelectrodes^{23,24}. The calculated value is close to zero membrane

potential because the external Na activity is close to one-twelfth of the internal K activity.

Figure 3b shows the voltage-dependence of the sodium permeability P_{Na} , calculated from the data in Fig. 3a by the following equation^{20,21}

$$P_{Na} = \frac{I_{Na}}{(Na)_0} \frac{RT}{EF^2} \frac{\exp(EF/RT) - 1}{\exp((E - E_{Na})F/RT) - 1} \quad (1)$$

where E is the membrane potential (inside minus outside), E_{Na} is the measured reversal potential, and R , T and F have their usual meanings. P_{Na} shows a sigmoid dependence with membrane potential, varying from almost no activation at -50 mV to nearly full activation at 0 mV. The smooth curve is drawn for half maximal activation at -26 mV and a maximal P_{Na} of $6.3 \times 10^{-5} \text{ cm s}^{-1}$. This estimate is close to that reported by Adrian and Marshall for rat skeletal muscle²⁵.

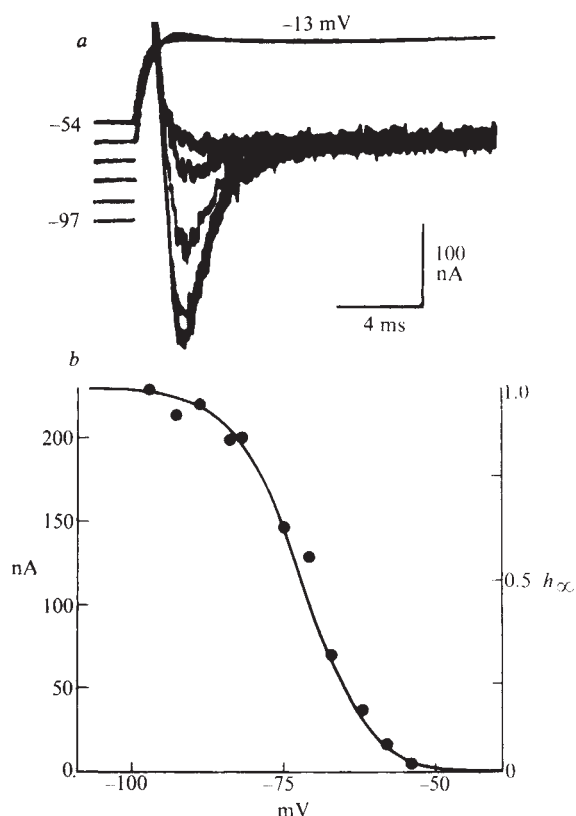


Fig. 4 Influence of membrane potential on I_{Na} inactivation. *a*, membrane currents elicited by depolarisations to -13 mV following 200-ms prepulses to -54, -62, -71, -80, -89 and -97 mV. Only the last 2 ms of the prepulse are shown. Peak currents following prepulses to -89 and -97 mV largely superimpose. *b*, Sodium inactivation curve obtained by plotting the amplitude of the inward current (corrected for capacity transient) against prepulse potential. The normalised data were fitted by the equation $h_\infty = 1/(1 + \exp(E + 72/5.45))$. Holding and test potential, -13 mV; pulsing rate, 0.2 Hz. External cylindrical surface area, 0.0024 cm^2 . Fibre T42-3, 5.4 mM Ca, 22 °C.

The phasic nature of the sodium permeability is attributed to a process termed sodium inactivation²⁶. Figure 4a shows one method for studying the voltage-dependence of inactivation. I_{Na} was elicited by step depolarisations to a fixed test potential (-13 mV). The test pulse was preceded by 200 ms prepulses to various voltage levels. As the current records indicate, the amplitude of I_{Na} was largest following a prepulse to -97 mV, while almost no sodium current was seen following a prepulse to -54 mV. The analysis of these data and others from the same

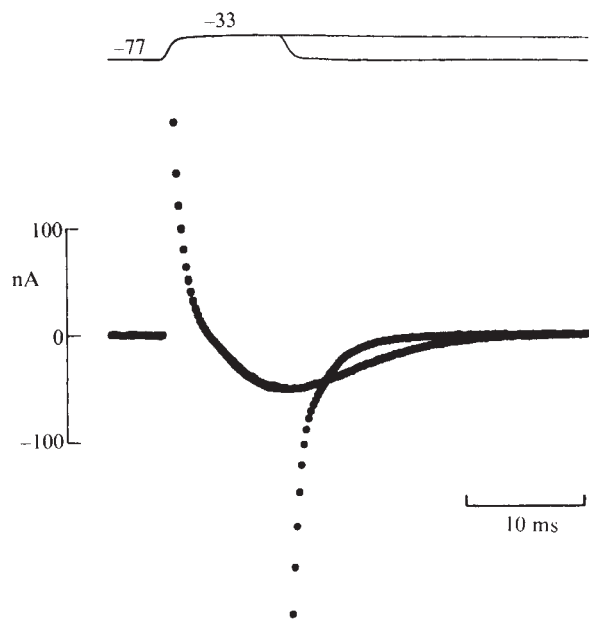


Fig. 5 Test of voltage control during I_{Na} . Superimposed records of recorded potential (top) and membrane current (bottom) during long and short clamp pulses. Brief pulse was terminated at peak P_{Na} . Good voltage control is indicated by the rapid decay of inward current following repolarisation. Traces are the average of two sweeps. Fibre T46-1.

experiment is shown in Fig. 4b. The amplitude of the I_{Na} transient displays a sigmoid dependence on prepulse potential. After normalisation, the data were fitted by the function

$$h_{\infty} = \frac{I_{Na}}{(I_{Na})_{\max}} = \frac{1}{1 + \exp((E - E_h)/k)} \quad (2)$$

where $E_h = -72$ mV and $k = 5.45$ mV. Table I presents data from a total of five experiments where $E_h = -74 \pm 4$ mV and $k = 5.36 \pm 0.71$ mV (mean $\pm$ s.e.m.). Equation (2) and very similar parameter values were used by Weidmann¹⁹ in his studies using maximal upstroke velocity as an index of P_{Na} . Our results resemble Thompson's data for rabbit Purkinje fibres in the double sucrose gap¹⁷, but differ from observations of Dudel and Rüdel in sheep Purkinje fibres²⁷. At 20 °C, Dudel and Rüdel found complete inactivation ($h_{\infty} = 0$) for potentials positive to -90 mV. We have seen similar behaviour in three exceptional cases in which the full inactivation curve was not determined. The data in Table I are representative of over 90% of our preparations, in which substantial I_{Na} is available at -85 mV, the normal resting potential at physiological temperature¹⁶.

The current signals in these experiments satisfy several criteria for adequate voltage control¹⁻⁸: (1) the inward current record shows a smooth decay, with no secondary humps or 'abominable notches' (Fig. 1); (2) the time course of the current remains unchanged when its magnitude is varied by prepulse inactivation (Fig. 4a); (3) the measured reversal potential lies close to the theoretically expected value (see ref. 5); and (4) activation is a gradual function of membrane potential, extending over a range of about 50 mV (Fig. 3b) as in other excitable tissues. There is no indication of the sharp voltage-dependence predicted for certain conditions where voltage control is poor^{2,6}.

Figure 5 shows an additional test for adequate clamp control which has been proposed in experimental^{14,17,28} and theoretical⁴ studies. The depolarising command pulse is terminated just as P_{Na} reaches its peak. If the membrane potential is adequately controlled, the 'tail' current should follow a smooth, rapid decay; if not, the current may be hooked or show the waveform of an inverted action potential. As Fig. 5 indicates, the experimental trace does in fact show a fast shut-off following the repolarisation.

Experimental evidence for adequate voltage control is compatible with rough calculations based on electrical properties of the preparation. Previous experiments¹⁶ provide an upper estimate of the effective external series resistance, $R_s \leq 3.3 \Omega \text{ cm}^2$. With this value, the voltage drop along R_s can be calculated for the maximum I_{Na} in Fig. 3 (120 nA) and the external surface area of the preparation (0.0032 cm²). Thus $(120 \text{ nA})(3.3 \Omega \text{ cm}^2)/(0.0032 \text{ cm}^2) = 0.12$ mV. This radial voltage error is negligible. Longitudinal voltage nonuniformity may be calculated by taking an experimental estimate of the longitudinal resistivity, $R_l = 450 \Omega \text{ cm}$. For the measured radius of 80 μm , the axial resistance per unit length is $r_l = 2.17 \text{ M}\Omega \text{ cm}^{-1}$. The negative slope region of the I - V relationship in Fig. 3 corresponds to a membrane resistance per unit length of $r_m = -6.3 \text{ k}\Omega \text{ cm}$. From these values, one calculates a longitudinal space constant $|\lambda| = (|r_m|/r_l)^{1/2} = 540 \mu\text{m}$. This estimate of $|\lambda|$ is long relative to the distance from the end of the cable to the current source (315 μm). At worst, when the potential at the end of the cable is near maximum inward current, the calculated voltage difference from end to current source is only -1.7 mV. Thus, the longitudinal nonuniformity should introduce relatively little distortion in the measured characteristics.

The success of these experiments is not restricted by the properties of the microelectrodes. Current passing limitations were not a problem during the surge of P_{Na} because of the small preparation size and the low sodium concentration. The speed of clamp control was mainly limited by the product of axial resistance and membrane capacitance. Thus, at strong depolarisations, the capacity transient seriously overlaps I_{Na} activation. For analysis of activation kinetics over a wide voltage range, or effects of intracellular constituents, the method of Lee *et al.*²⁹ is clearly preferable. Our approach is very suitable, however, for analysis of sodium permeability over the voltage range where action potentials are initiated. It offers the possibility of studying sodium channels and their pharmacological modification in intact cardiac tissue free from enzymatic treatment. Further work will be needed to establish how cardiac sodium channels differ from those in other excitable membranes.

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Sodium current in single heart muscle cells

A COMPLETE description of the sodium current, I_{Na} , in heart muscle has not been obtained despite the practical importance of such information as a rational basis for the therapy of arrhythmias. I_{Na} has been examined under voltage clamp using micropipette¹ or sucrose gap methods², but the adequacy of spatial control of voltage has been questioned and temporal resolution of the early kinetics has been unsatisfactory and results therefore inconclusive³. These problems reflect the fact that heart muscle is a syncytium with restricted extracellular compartments. One of the more favourable preparations is rabbit Purkinje fibres⁴ and Colatsky and Tsien⁵ have recently performed voltage clamp studies on them with the two micropipettes technique. They have demonstrated the feasibility of this approach when proper precautions are taken⁵. Quite a different approach has been taken by other investigators such as Mehdi and Sachs⁶ and Powell *et al.*⁷⁻⁹, who have dispersed individual heart muscle cells and examined them with micropipette techniques. In this way the complex geometry is greatly simplified although the enzymes used in dispersion may pose another set of problems. We have followed this second approach and have isolated individual heart muscle cells from adult rats with a modified version of the dispersion method reported by Powell and Twist⁹. To perform electrophysiological studies we have sucked single cells into a suction pipette and used a technique which allows voltage clamp and internal perfusion¹⁰. I_{Na} has been isolated by suppression of other cardiac currents and its kinetics separated from the capacitive current transient. The success of the present approach indicates its potential usefulness in cardiac electrophysiology.

The suction pipette method is similar to that used in snail neurones and the details have been published elsewhere¹⁰. The main difference in the present experiments is that the tip diameter of the suction pipette was smaller, about 20 μm , and the measured tip resistances ranged from 150 to 250 k Ω . Series resistances determined from the peak amplitude of the capacitive current transient have similar values, indicating that the

tip resistance was mainly responsible. For currents less than $\sim 2 \times 10^{-8}$ A, the effects were negligible but for larger currents series resistance compensation¹⁰ was used. The rise time of the voltage clamp step was 10 μs and the capacitive current transient had a single time constant 100–200 μs in duration. Current–time records during single clamp runs were digitised every 5 μs using a 12-bit digital oscilloscope with a 2 K memory (Nicolet 575) and stored on a digital tape recorder (Kennedy Model 9700). Currents produced by voltage steps equal in amplitude but opposite in sign could be added to remove leakage and shunt currents and the linear components of the capacitive current. The external solution for separating I_{Na} had the following composition in mM: sodium isethionate, 120; CsF, 5.4; Mg_2SO_4 , 2.85; NaH_2PO_4 , 0.42; NaHCO_3 , 25; glucose, 5.5; pH 7.5 when equilibrated with 95% O_2 , 5% CO_2 . In the early experiments 5.0 mM tetraethylammonium was added to both internal and external solutions but later its use was discontinued due to its lack of effect. Cs^+ was used to substitute for K^+ in the internal solution which had the following composition (mM): CsF, 130; NaHCO_3 , 16; glucose, 5.5, pH 7.3 when equilibrated with 95% O_2 and 5% CO_2 . Experiments were carried out at 23°C. The general procedures for obtaining individual, isolated beating adult rat myocytes have been described⁹ and details as well as biochemical characteristics of the cells prepared in this way will be published elsewhere.

The structural characteristics of single dispersed myocytes are similar to those already reported^{11,12}. The individual cells are separated at the intercalated disks, are approximately cylindrical, but have longitudinal grooves and transverse ridges with T-tubule openings near these ridges. Cell dimensions were 15–25 μm radius and about 100 μm length. Under the dissecting microscope the cells were beating in solution before and after aspiration, but during maintained suction visible beating ceased. In low or zero Cl^- solutions resting potentials were –70 to –90 mV (ref. 7) and the cardiac action potential had the shape shown in Fig. 1a. The short duration of the plateau may be related to the rapid heart rate of the rat (4 Hz); similarly shaped action potentials for intact rat ventricle¹³ and dispersed rat myocytes⁸ have been reported. Potassium currents were suppressed by Cs^+ substitution for internal and external K^+ , Ca current was suppressed either by Co^{2+} and/or zero Ca^{2+} solutions and Cl^- current was suppressed by isethionate[–] and F^-

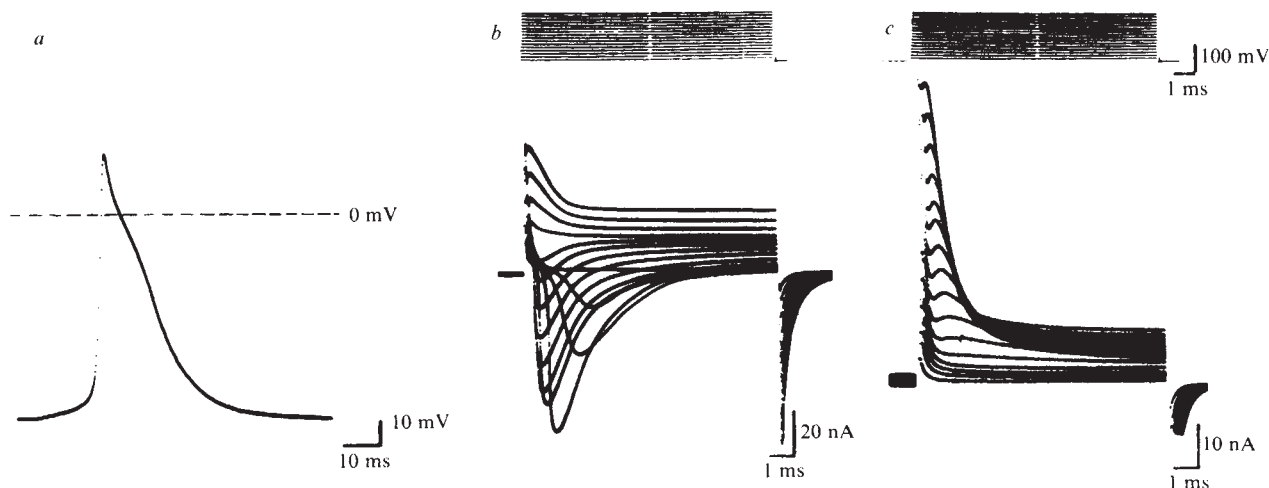


Fig. 1 Membrane potential and I_{Na} in single rat heart muscle cells. Individual myocytes were obtained by exposing rat ventricles to Krebs–Henseleit solutions containing 0.1% collagenase (Sigma type 1) and 0.1% hyaluronidase (Sigma type 1) according to the methods of Powell *et al.*⁷⁻⁹. *a*, Record of an action potential obtained from a single rat heart muscle cell in zero Cl^- solutions using the suction pipette method. Stimulating current was delivered by current clamp. The resting potential is –80 mV. The action potential amplitude is about 100 mV with a 20 mV overshoot and was corrected for the voltage drop across the tip resistance. The plateau phase is shortened and similar configurations in rat heart muscle have been reported^{8,13}. *b*, *c*, I_{Na} records uncorrected for capacitive, leakage and shunt currents. I_K , I_{Ca} and I_{Cl} have been suppressed by substituting Cs^+ for K^+ , Co^{2+} for Ca^{2+} and isethionate[–] and F^- for Cl^- . The traces are from the same cell in 100% (*b*) and 0% (*c*) external Na solutions. In (*b*) I_{Na} is resolved after about 150 μs and a clear reversal of currents is observed. In (*c*) only outward current appears. In both cases, the peak inward or outward current appears and then disappears within the first 2–6 ms depending on the magnitude of the step voltage change. As the step potential becomes progressively higher, peak current appears more quickly. Holding potential, $V_H = -90$ mV. $1-2 \times 10^{-9}$ A of inward current was usually required to achieve this V_H . Each voltage trace represents a 10 mV increment from V_H .

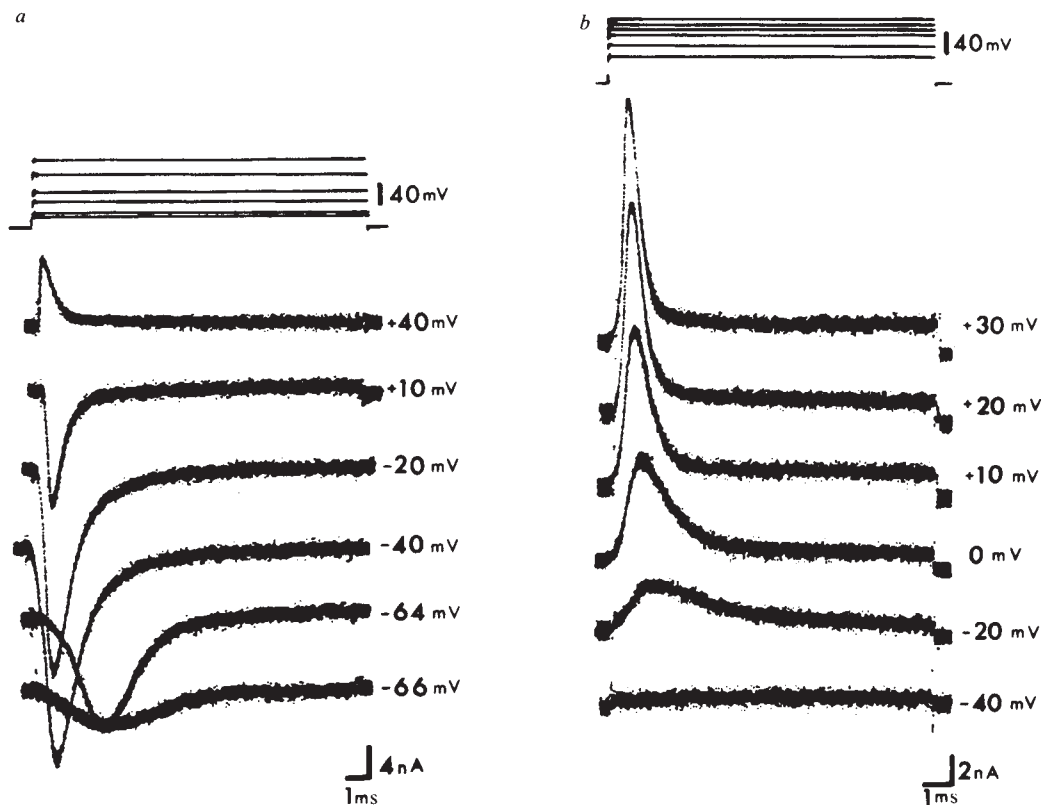


Fig. 2 I_{Na} currents after subtraction of leakage, shunt and linear capacitive currents and their I - V relationships. *a*, I_{Na} values from a single myocyte in 50% $[Na^+]_o$. *b*, Same cell in zero Na^+_o . $V_H = -90$ mV and corresponding clamp potentials are indicated besides each current trace. Activation and inactivation are apparent and are faster at higher voltages. A small outward current persists in zero Na^+_o solutions. The reason for the slower kinetics in (*b*) is explained in the text.

substitution. After suppression of I_K , I_{Ca} and I_{Cl} , the current-time records and associated clamp potentials in 100% Na solutions are shown in Fig. 1*b*. The currents in these records have deliberately not been corrected for leakage or capacitive components and show that early I_{Na} is clearly distinguishable from the capacitive transients. In the photographic reproductions the latter appear mainly as discontinuities after the step depolarisations. As voltage amplitudes are increased above threshold the inward current becomes progressively larger and faster, but then the amplitude decreases as the voltage step approaches E_{Na} . The currents reverse as the steps go positive

with respect to E_{Na} . A small delay preceding activation is present and is more apparent in Fig. 2. I_{Na} also shows inactivation; this process is about 4–8 times slower than activation. After leakage subtraction it appears that inactivation of inward I_{Na} is complete. The tail currents occurring during repolarisation are capacitive and have a single time constant of 100–200 μ s which is similar to that measured at the onset of the step depolarisations. When Na^+ is completely replaced by Tris or isotonic sucrose solution, I_{Na} is outward only, as Fig. 1*c* demonstrates. In the absence of extracellular Na^+ , a small steady outward current persists and this current is often smaller at more

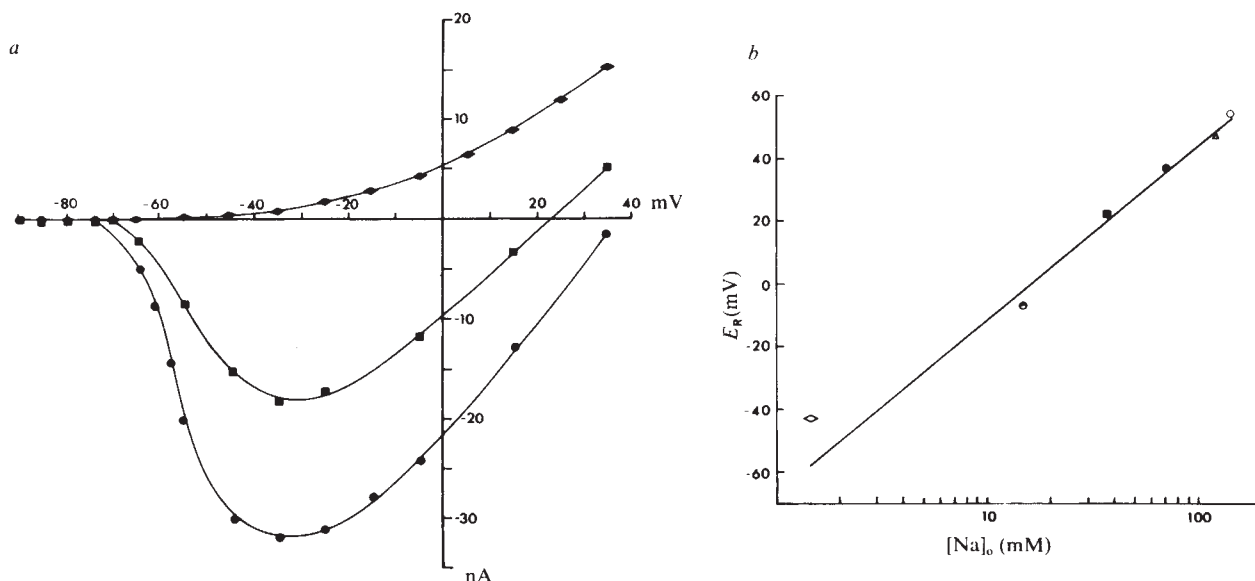


Fig. 3 Effects of changes in $[Na^+]_o$. *a*, Current-voltage relationship of peak inward and outward Na^+ currents in external solutions containing varying amounts of sodium as follows: 50% (●), 25% (■) and zero (◆). Same cell as in Fig. 2. The peak currents are sensitive to both voltage and $[Na^+]$. The null potential of this current shifts to the left along the voltage axes as $[Na^+]_o$ decreases and becomes totally outward as the sodium in the external solutions is reduced to zero. *b*, Changes of E_R in relation to log changes of $[Na^+]_o$. Each point is from a different myocyte. The solid line represents the Nernst potentials for $[Na^+]_i = 16$ mM, the internal perfusate concentration. Na^+_o concentrations are: 100% (○), 75% (△), 50% (●), 25% (■), 10% (◐) and 1% (◆).

positive voltages leading to a crossover of the steady outward currents at long times. This may reflect the voltage-dependence of the inactivation process but could also be due to a voltage-dependent blocking action of Cs^+ upon I_{Na} similar to that observed in squid axon¹⁴. When I_{Na} was outward, it could be abolished by replacing Na_i with Cs . The effect appeared 1 to 2 min after solution change and was complete after 10–20 min indicating that internal perfusion can effectively control intracellular ionic composition.

As Na^+ substitutes we used Tris or tetramethylammonium (TMA) ions or isosmotic replacement of NaCl with sucrose. TMA had deleterious effects within a few minutes and the cells did not tolerate exposures to Tris for periods longer than 15 min. At shorter times, however, Tris was a reasonable substitute. The effects of Tris or sucrose substitutions on I_{Na} were similar.

The current–voltage relationship in 100% Na_0 solutions often had a steep negative resistance region indicating inadequate voltage control. This problem seldom occurred when $[\text{Na}^+]_0$ was reduced to 50% or less as shown in Fig. 3a. The relationship between peak I_{Na} and voltage in the different solutions was derived from traces of currents such as those shown in Fig. 2 which were obtained after subtraction of leakage and linear capacitative components. Lowering $[\text{Na}^+]_0$ did not appear to alter the basic Na^+ conduction mechanism. Thus, in low Na^+ solutions currents were reduced in accordance with the independence principle and kinetics and normalised conductance–voltage relationships were similar. The slower kinetics in zero Na_0^+ (Fig. 2b) compared to $1/2 \text{ Na}_0^+$ (Fig. 2a) reflect the effects of experimental times greater than 30 min which were required to complete the studies in several different external solutions. Similar changes may also occur at prolonged times in a single test solution. We used chord conductances for conductance calculations because the instantaneous I – V curves were linear. The G_{Na} – V curve at its steepest increased e -fold for about 10 mV of depolarisation, which is similar to the findings of Colatsky and Tsien⁵. I_{Na} could also be inactivated by depolarising prepulses and the extent of inactivation was determined by the duration and amplitude of the prepulse.

The reversal potentials, E_r 's, for leakage-corrected I_{Na} are similar to those predicted by the Nernst potentials for the internal and external concentrations of Na^+ used (Fig. 3b); this indicates a high selectivity of the conductance for sodium ion.

The degree of complexity of membrane geometry in the present preparation needs to be examined in detail because a major weakness in voltage clamp studies of heart muscle has been an inadequate consideration of such complications³. Towards this end impedance studies are now underway. Calculations of surface area derived from the capacitative current transient are slightly larger than calculations assuming a right cylinder of the dimensions referred to but some of the discrepancy might be due to the longitudinal grooves and transverse ridges present in these cells. A contribution from T-tubules is also possible, but a complicated equivalent electrical circuit may be unlikely because the capacitative current transient is a single exponential function. Spatial control of voltage seems adequate as there are no notches on the current records, the negative slope region of the I – V curves in solutions with 50% Na or less is not overly steep, that is, peak inward current increases gradually with voltage in this range and lowering $[\text{Na}^+]_0$ reduces currents without altering kinetics or voltage-dependence. With the present method it is possible to examine I_{Na} in the outward direction only and avoid negative resistances where voltage control is most difficult (Fig. 3a, zero Na_0^+ curve). In three instances, we were able to compare recorded values for the maximum rate of rise of the action potential dV/dt_{max} , with computed values using I_{Na} and C_m calculated from the capacitative current transient, and these were in reasonable agreement. As all other currents were suppressed it cannot be concluded, however, that under normal circumstances dV/dt_{max} and I_{Na} would be equivalent.

The effects of enzyme treatment upon I_{Na} require some comment. The high selectivity of G_{Na} and the similarity of our

G_{Na} – V curve to the P_{Na} – V relationship obtained by Colatsky and Tsien⁵ in the absence of enzymes suggest that enzyme treatment has few effects. Moreover, collagenase treatment of mammalian skeletal muscle had little or no effect on resting or action potentials¹⁵.

In *Helix* neurones, the enzyme trypsin does not alter the electrical properties of I_{Na} but it does abolish tetrodotoxin (TTX) sensitivity¹⁶. We found that myocyte I_{Na} was blocked by TTX but unlike axons, the blockage requires micromolar concentrations and is voltage-dependent, being less complete at more positive potentials. A voltage-dependent action of TTX on dV/dt_{max} in untreated guinea pig papillary muscle cells has been reported¹⁷ as having the large concentration requirements. Therefore the presently reported TTX effects are probably not due to enzymatic actions but result either from differences in all Na channels, or a small number of TTX-resistant channels.

A full description of I_{Na} is clearly feasible as the present report and that of Colatsky and Tsien⁵ indicate. Our methods also provide the capability for simultaneous voltage clamp and internal perfusion of individual cardiac cells. This approach will be very useful for future electrophysiological studies on heart muscle and similar approaches are likely to be successful in other body tissues.

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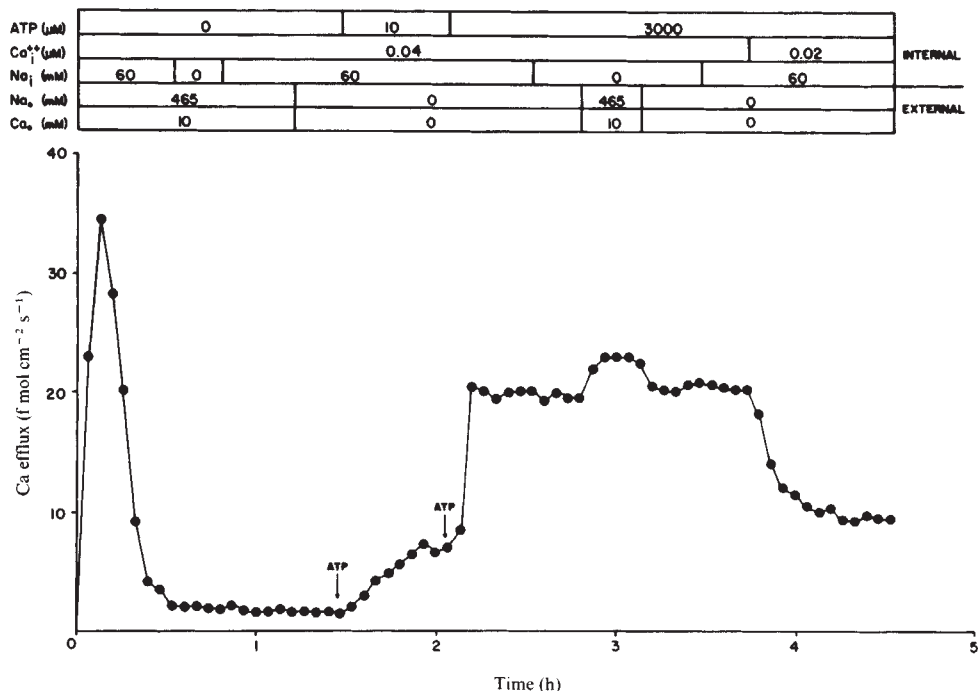
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Physiological role of ATP-driven calcium pump in squid axon

IT has been proposed that the regulation of the ionised calcium concentration inside many excitable and non-excitable cells is mediated by a Na – Ca countertransport mechanism where the Na electrochemical gradient provides the energy to maintain low levels of intracellular Ca^{2+} (refs 1, 2). Two recent observations, however, provide evidence consistent with the idea that Ca extrusion in squid axons is mediated, at least in part, by an ATP-driven Ca pump of the type observed in red blood cells^{3,4}. First, in the absence of a Na gradient across the membrane there is an ATP-dependent 'uphill' extrusion of Ca which persists in the absence of external Na , Ca and Mg (ref. 3). And second, in Ca -injected unpoisoned axons, 50–90% of the Ca efflux can occur as an 'uncoupled' flux—that is, Ca efflux not accompanied

Fig. 1 Effects of Na_i , Na_o and ATP on the activation of Ca efflux at physiological Ca_i^{2+} concentration (axon 010678A, 530 μm). The composition of the dialysis solution was (mM): K^+ 310; Na^+ 60; Mg^{2+} 4 in excess of the ATP concentration; Tris $^+$ 5; Cl^- 79; aspartate 310; EGTA 1; glycine 330; oligomycin 10 $\mu\text{g ml}^{-1}$. The osmolality was 980 mosM and the pH (17°C) 7.1. ATP and phosphoarginine (PA) were obtained from Boehringer as sodium salts and were stored at -90°C as stock solutions (250 mM at pH 7.1). The ATP solution contained in addition 250 mM MgCl_2 . The composition of the standard artificial seawater was (mM): Na^+ 440; K^+ 10; Mg^{2+} 50; Ca^{2+} 10; Tris $^+$ 10; Cl^- 580; EDTA 0.1. The osmolality was 1,000 mosM and the pH (17°C) 7.6. Tris $^+$ was used as Na^+ substitute. Removal of Na_i or Na_o and Ca_o was carried out without changing the other constituents. The nominally Ca-free media contained 0.5 mM EGTA and no added Ca. The axon was preincubated for 1 h in KCN before dialysis. At zero time, the dialysis began with the indicated solution plus radioactive Ca. The fluid superfusing the axon (1 ml min^{-1}) contained nominally 1 mM KCN and was collected at 4-min intervals. An appropriate volume of a scintillation mixture was added and the samples counted in a scintillation counter. The reduction following the initial rise in Ca efflux is a consequence of the ATP washout. The arrows refer to addition of ATP + PA to the internal medium.



by the uptake of Na, Ca or Mg (ref. 5). The existence of two apparently separate mechanisms for Ca transport, Na-Ca exchange and an ATP driven Ca-pump, raises the question of whether, in normal conditions, both mechanisms participate in the maintenance of the physiological $p\text{Ca}$. To be of any use in controlling the $p\text{Ca}$ of the nerve, the mechanism responsible must be able to operate at normal Ca^{2+} concentrations, $<10^{-7}\text{M}$ (ref. 6). We now present evidence showing (1) that in squid axons with physiological levels of Ca_i^{2+} most of the Ca efflux can be accounted for by an ATP-dependent system with high affinity for Ca_i^{2+} and ATP and which can operate in the complete absence of external Na and Ca; and (2) that a Ca transport mechanism with a low affinity for Ca_i^{2+} and ATP and extremely sensitive to Na_i , Na_o and Ca_o can contribute substantially to the total Ca efflux only at Ca_i^{2+} well above the physiological Ca^{2+} concentrations.

The present experiments were carried out on giant axons of the squid *Loligo pealei*, using the internal dialysis technique to control the intracellular concentration of low molecular weight solutes⁷. Washout of internal ATP was achieved using glass dialysis capillaries. The amount of ADP produced by hydrolysis of exogenous ATP was minimised by adding 5 mM phosphoarginine to the dialysis solutions⁸.

The dependence of Ca efflux on Na_i , Na_o and ATP was determined at a Ca_i^{2+} concentration of 0.04 μM (physiological levels of Ca_i^{2+} are between 0.02 μM and 0.1 μM)⁵. After the washout period a steady Ca efflux of $\sim 2\text{ fmol cm}^{-2}\text{ s}^{-1}$ was obtained (Fig. 1). Changes in the Na electrochemical gradient (removal of internal Na at constant external Na and vice versa) had no effect on Ca efflux. In contrast, addition of as little as 10 μM ATP, in the absence of Na_o and Ca_o , led to a significant increase in Ca efflux. In the presence of 3 mM ATP removal of Na_i had no effect on Ca efflux, whereas readdition of external Na and Ca increased Ca efflux slightly and reversibly. Note also that the ATP dependent Ca efflux can operate at very low Ca_i^{2+} , as the levels of Ca efflux at 0.02 μM Ca_i^{2+} are appreciably higher than the basal Ca efflux observed in the absence of ATP (Fig. 1).

Figure 2 shows that the ATP dependent, Na_o and Ca_o independent (uncoupled) Ca efflux constitutes most of the Ca efflux at physiological Ca_i^{2+} concentrations, with a $K_{1/2}$ for Ca_i^{2+} of $\sim 0.18\text{ }\mu\text{M}$. On the other hand, the ATP independent, Na_o

and Ca_o dependent Ca efflux is minimal at physiological Ca_i^{2+} concentrations and shows no sign of saturation up to $\sim 1\text{ }\mu\text{M}$ Ca_i^{2+} . When internal ionised calcium was raised to 80–110 μM , the Na_o dependent Ca efflux rose to 680–750 $\text{fmol cm}^{-2}\text{ s}^{-1}$ and was highly sensitive to internal Na concentration (data not shown). In *Dorytheutis plei*⁹ at physiological Ca_i^{2+} and Na_i^{+} concentrations, $>95\%$ of the Ca influx was independent of Na_i and Na_o and increased linearly with the external Ca concen-

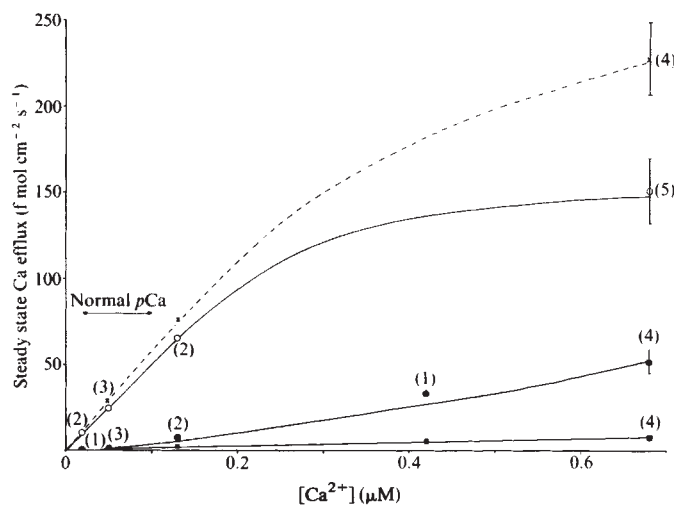


Fig. 2 Effect of internal ionised Ca on the Ca efflux components in dialysed squid axons. $\text{Na}_i = 65\text{ mM}$; temperature 16.5°C . $\times$, Ca efflux measured in ATP-dialysed axons (3 mM ATP + 5 mM PA) bathed in standard artificial seawater. $\circ$, Ca efflux measured in ATP-dialysed axons in solutions with Na_o and Ca_o both zero ('uncoupled' component). $\bullet$, Na-Ca dependent Ca efflux measured in ATP-free axons. $\blacksquare$, Ca efflux measured in ATP-free axons with Na_o and Ca_o both zero (leak component). Ionised calcium in the dialysis medium was controlled by varying the ratio (CaEGTA)/(free EGTA) at a constant total EGTA of 1 mM. The apparent dissociation constant for the CaEGTA complex was chosen as 0.15 μM ⁵. The numbers above the experimental points represent the number of determinations. The physiological range of Ca_i^{2+} concentration is indicated by the horizontal arrow.

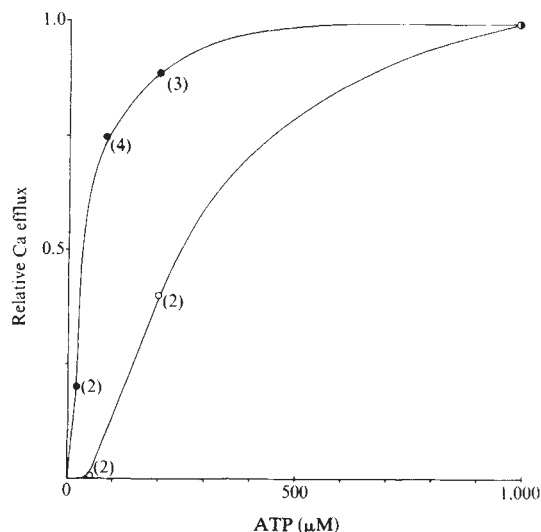


Fig. 3 Effect of internal ATP on the 'uncoupled' and Na-Ca dependent components of the Ca efflux. Ordinate: Ca efflux relative to the maximum efflux obtained with 1 mM ATP. Abscissa: ATP concentration in the dialysate in μM . The ATP in the axoplasm was buffered by adding 5 mM phosphoarginine to the dialysis medium. ●, Steady state Ca efflux level in the absence of external Na and Ca ('uncoupled'). ○, Na-Ca dependent Ca efflux. Half activation was obtained at 30 μM and 230 μM respectively. In most of the experiments shown above, the ATP dependence of both components was tested in the same axon. Once the ATP from the fibre had been removed by dialysis (washout 1–2 h) Na and Ca were removed from the external medium and increasing amounts of ATP (10, 50, 200, 1,000 μM) were sequentially added to the dialysis fluid. Once the ATP dependent 'uncoupled' Ca efflux had reached a steady state corresponding to each ATP concentration, the external Na and Ca sensitivity was tested in square pulse fashion. The number to the right of the experimental points represent the number of determinations. The curves were drawn by eye. The mean temperature was 17 °C.

trations. With 3–4 mM external Ca the influx of Ca was $\sim 38 \text{ fmol cm}^{-2} \text{ s}^{-1}$; this 'leak' influx closely matches the magnitude of the uncoupled Ca efflux observed here and obtained in comparable conditions. With high Ca_i^{2+} concentrations Ca influx was mainly through the Na-Ca exchange mechanism⁹.

Although ATP is not essential for the Na-Ca exchange mechanism to operate, it is known that the addition of ATP brings about an increase in the rate of that exchange¹⁰. Figure 3 shows several experiments where the relative rate of the 'uncoupled' Ca efflux and the ATP-stimulated Na-Ca exchange are plotted as a function of the ATP concentration in the dialysis solution. It is clear that the 'uncoupled' component shows a much higher affinity for ATP ($K_{1/2} = 30 \mu\text{M}$) than the Na-Ca exchange ($K_{1/2} = 230 \mu\text{M}$).

The experiments described above indicate the existence of two components of Ca efflux in squid axons: (1) uncoupled Ca efflux and (2) Na-Ca exchange. The properties of these components suggest that they indeed represent two different mechanisms for Ca translocation.

The 'uncoupled' Ca efflux, which has all the properties of a Ca pump, operates in the physiological range of Ca_i^{2+} concentration, thus playing a fundamental part in the regulation of intracellular ionized calcium. The Na-Ca exchange becomes more effective when Ca_i^{2+} is increased far beyond the physiological range. Although ATP hydrolysis is almost certainly involved in the operation of the Ca pump, the role of ATP on the Na-Ca exchange is still not clear. It is possible that the high Ca affinity (Ca + Mg)ATPase described by Robinson¹¹ in rat brain microsomes represents the biochemical counterpart of the ATP-fuelled Ca-pump considered here.

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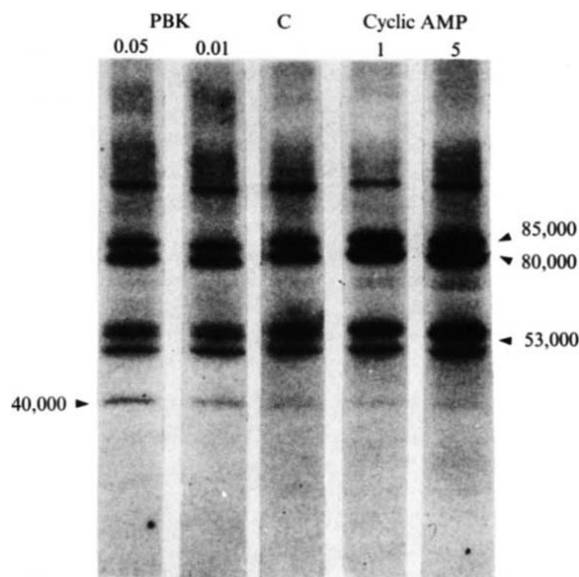
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Phosphorylase kinase phosphorylates a brain protein which is influenced by repetitive synaptic activation

SYNAPTIC systems from mammalian brain can now be studied *in situ* using a brain slice preparation which displays most of the electrophysiological characteristics observed in intact preparations^{1,2}. We have been particularly interested in analysis of long-term potentiation (LTP), a marked and quasi-permanent increase in synaptic efficacy which is found in the hippocampal formation following a brief (1 s) train of repetitive stimulation^{3–6}. Previous work from this laboratory demonstrated a strong correlation between induction of such LTP and the apparent phosphorylation of a 40,000 molecular weight protein in synaptic plasma membranes (SPM)^{7,8}. Both LTP and the stimulation-dependent changes in the 40,000-MW protein were dependent on calcium and this cation has also recently been shown (ref. 9 and unpublished observations) to enhance the endogenous phosphorylation of a protein with a molecular weight of $\sim 40,000$. If potentiation and the phosphorylation of the 40,000-MW protein are related, with calcium being required for both, then it is possible that a calcium-dependent protein kinase might be an intermediary enzyme in the two processes. We have therefore investigated the effect of exogenous phosphorylase kinase (PBK; EC 2.7.1.38), a calcium-sensitive enzyme found in brain^{10,11}, on the phosphorylation of the 40,000-MW protein. Since several laboratories have demonstrated cyclic AMP-dependent phosphorylation of several SPM proteins^{12–15}, it was also of interest to test for the possibility that this nucleotide might be involved in the phosphorylation of the 40,000-MW protein. We report here that PBK produces a marked and highly specific phosphorylation of the 40,000-MW protein that we have previously shown to be affected by high frequency potentiating stimulation.

We assayed the endogenous phosphorylation (in the presence of either cyclic AMP or PBK) of a SPM fraction which was prepared from hippocampal slices maintained in conditions described previously². Figure 1 shows an autoradiograph from a typical experiment. As can be seen, PBK markedly stimulated the phosphorylation of the 40,000-MW protein and had no detectable effect on any other protein band. Cyclic AMP produced a significant stimulation of three bands with MWs of 85,000, 80,000 and 53,000, as has been reported by several investigators^{12–15}, but it had no effect on the phosphorylation of

Fig. 1 Autoradiograph showing the effects of PBK and cyclic AMP on the endogenous phosphorylation of SPM proteins. PBK produced a marked dose-dependent increase, compared to control (C), in the phosphorylation of the 40,000-MW protein. The final concentrations of PBK were 0.01 and 0.05 mg ml⁻¹. Quantification was based on densitometric scans as described previously⁸. Here, 0.01 mg ml⁻¹ PBK increased the phosphorylation of the 40,000-MW protein by 45% and 0.05 mg ml⁻¹ increased the phosphorylation by 140%. Cyclic AMP produced significant stimulation of the phosphorylation of proteins with MWs of 85,000, 80,000 and 53,000, but this nucleotide had no effect on the phosphorylation of the 40,000 protein. The concentrations of cyclic AMP used in these experiments were 1 and 5 μ M. Molecular weight estimates were based on comparisons with the mobilities of standards of known MW. The SPM fractions used were prepared⁸ from hippocampal slices which had been maintained *in vitro*² for ~1 h. After isolation SPMs were preincubated at 30 °C in 40 μ l of the assay buffer (10 mM MgCl₂, 50 mM HEPES, pH 7.4), at a protein concentration of 0.25 mg ml⁻¹, and after 5 min the reaction was initiated by the addition of 10 μ l of [γ -³²P]ATP (10 μ Ci, 20 μ M final concentration) and was terminated after 20 s by the introduction of 30 μ l of a stop solution which yielded concentrations of: 2.3% SDS (w/v), 5% β -mercaptoethanol (v/v), 62.5 mM Tris HCl, 10% glycerol, pH 6.8. Samples were heated in a boiling water bath for 3 min and then run on SDS-polyacrylamide gradient gels¹⁶. The gels were stained for protein with Coomassie blue, dried under vacuum and exposed to Kodak RP Royal X-Omat film for 3–4 d. Kinematic studies, to be described elsewhere, indicate that incorporation of label in this assay reflects the initial velocity of the phosphorylation reaction. Cyclic AMP and phosphorylase kinase (Sigma, in 5 μ l assay buffer) were introduced just before addition of ATP to lessen possible phosphodiesterase attack on the cyclic AMP or proteolytic degradation of the phosphorylase kinase. Addition of 5 μ l of assay buffer just before addition of ATP had no effect on the phosphorylation of control samples. In the endogenous assay of the effects of PBK, no calcium is added but it can be assumed that endogenous calcium remains following the use of 50 μ M calcium in our fractionation solutions. The question of whether calcium can stimulate the phosphorylation of the 40,000-MW protein is obviously important to our hypothesis that a stimulation-dependent calcium influx activates PBK which in turn phosphorylates the protein. Hershkovitz⁷ has shown, using Ca/EGTA buffers, that the phosphorylation of a 41–43,000-MW SPM protein is stimulated by calcium. Our preliminary experiments, also using Ca/EGTA buffers, indicate that the 40,000-MW protein is stimulated by but not totally dependent on calcium. It is important to note in this regard that although PBK has an almost absolute requirement for calcium in its phosphorylation of phosphorylase *b*¹⁰, PBK from tissue other than skeletal muscle retains a considerable proportion (25–40%) of its activity in the absence of calcium¹⁷; in the case of troponin, calcium enhances a basal level of PBL-catalysed phosphorylation of this contractile protein¹⁸.



the 40,000-MW component. It should be noted that several bands which undergo phosphorylation in our assay are apparently not significantly influenced by either phosphorylase kinase or cyclic AMP.

It is possible that the very specific effects of PBK could occur if the kinase were sterically hindered and hence did not have access to other substrates. We therefore tested for the effects of another soluble kinase, a cyclic AMP-dependent protein kinase from beef heart (Sigma), in this autoradiograph. In sample *a*, which contained the catalytic subunit at a concentration of 0.025 mg ml⁻¹, there was a significant stimulation compared to the control (*b*), of three bands with MWs 85,000, 80,000 and 53,000. Similarly the holoenzyme produced a large stimulation of these same bands and also increased the incorporation of ³²P into a band with a MW of 57,000. This latter band may be the regulatory subunit of the beef heart protein kinase which has been reported to undergo autophosphorylation. Densitometric quantification indicated that the catalytic subunit and the holoenzyme of cyclic AMP-dependent protein kinase produced increases in the phosphorylation of the 40,000-MW protein of less than 5% in this autoradiograph and similar values were obtained in five separate experiments. All enzymes were added just before addition of ATP and reaction conditions were identical to those in Fig. 1.

It seems then that PBK selectively phosphorylates an SPM protein which is also strongly influenced by repetitive synaptic

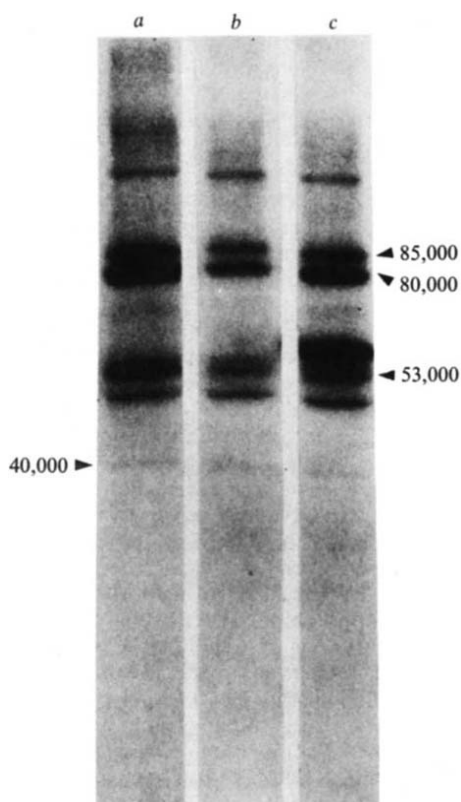


Fig. 2 The pattern of incorporation of ³²P into specific bands of a SPM fraction, assayed in the presence and absence of either the holoenzyme or the catalytic subunit of the cyclic AMP-dependent protein kinase from beef heart (Sigma), is shown in this autoradiograph. In sample *a*, which contained the catalytic subunit at a concentration of 0.025 mg ml⁻¹, there was a significant stimulation compared to the control (*b*), of three bands with MWs 85,000, 80,000 and 53,000. Similarly the holoenzyme produced a large stimulation of these same bands and also increased the incorporation of ³²P into a band with a MW of 57,000. This latter band may be the regulatory subunit of the beef heart protein kinase which has been reported to undergo autophosphorylation. Densitometric quantification indicated that the catalytic subunit and the holoenzyme of cyclic AMP-dependent protein kinase produced increases in the phosphorylation of the 40,000-MW protein of less than 5% in this autoradiograph and similar values were obtained in five separate experiments. All enzymes were added just before addition of ATP and reaction conditions were identical to those in Fig. 1.

activation. Further evidence that these two treatments are acting on the same polypeptide was obtained in experiments in which endogenously phosphorylated SPM proteins were analysed in the two-dimensional gel system of O'Farrell¹⁹. As is apparent in Fig. 3, the 40,000-MW material migrates as one (and sometimes two) quite basic polypeptides, and this material shows increased incorporation following the addition of PBK. This very basic spot (or doublet) represents essentially all of the phosphoprotein material found in the two-dimensional gel at the 40,000-MW position. These data indicate that PBK probably phosphorylates the same protein which shows changes in endogenous phosphorylation following repetitive synaptic stimulation, as the kinase increases the phosphorylation of all the 40,000-MW material resolved in the two-dimensional gel analysis. Furthermore, in preliminary studies (nine separate experiments) we have found that repetitive synaptic stimulation reduces the subsequent PBK-induced phosphorylation of the 40,000-MW band.

Studies of protein phosphorylation in brain often concentrate on cyclic AMP-dependent phosphorylation, but our data

suggest that this nucleotide is not involved in the change in the phosphorylation of the 40,000-MW protein that follows synaptic activation. We propose that the change produced in the phosphorylation of the 40,000-MW protein is more likely to be mediated by activation of phosphorylase kinase. This activation could result from the intraterminal accumulation of Ca^{2+} that is thought to occur during high frequency stimulation²⁰⁻²³. Support for this hypothesis can be found in recent work which provides evidence that brain phosphorylase kinase is activated following stimulation^{24,25}, during seizures²⁶, and following application of putative neurotransmitters to cortical brain slices²⁷. Such an hypothesis obviously necessitates a function for phosphorylase kinase in brain other than its classical role in glycolysis, as the substrate for this process (phosphorylase *b*) has a MW considerably larger than the 40,000-MW protein. However, there is considerable evidence which indicates that PBK may in fact have several roles. Although controversial²⁸, England *et al.*²⁹ reported that the PBK-catalysed phosphorylation of troponin influences the Ca^{2+} sensitivity of the actomyosin ATPase. Interestingly, the troponin-T subunit, which is preferentially phosphorylated by PBK¹⁸, closely resembles the 40,000-MW SPM protein in MW and in its behaviour in isoelectric focusing gels³⁰. PBK has also been shown to modulate Ca^{2+} transport in the sarcoplasmic reticulum^{31,32} and the plasma membrane of skeletal muscle³³, but no protein substrate has yet been identified in this action although phosphorylase *b* inhibits the effect^{31,32}. Processes such as these are particularly exciting as potential modes of action for PBK in brain, especially in view of the possibility that the activation of PBK underlies the phosphoprotein changes correlated with long-term potentiation.

The relationship between the 40,000-MW protein changes produced by electrical stimulation and the extraordinarily long lasting increase in synaptic strength which follows such stimulation is uncertain. However, our demonstration that PBK selectively phosphorylates the 40,000-MW protein suggests new avenues for investigating the biochemical substrates of synaptic plasticity in brain.

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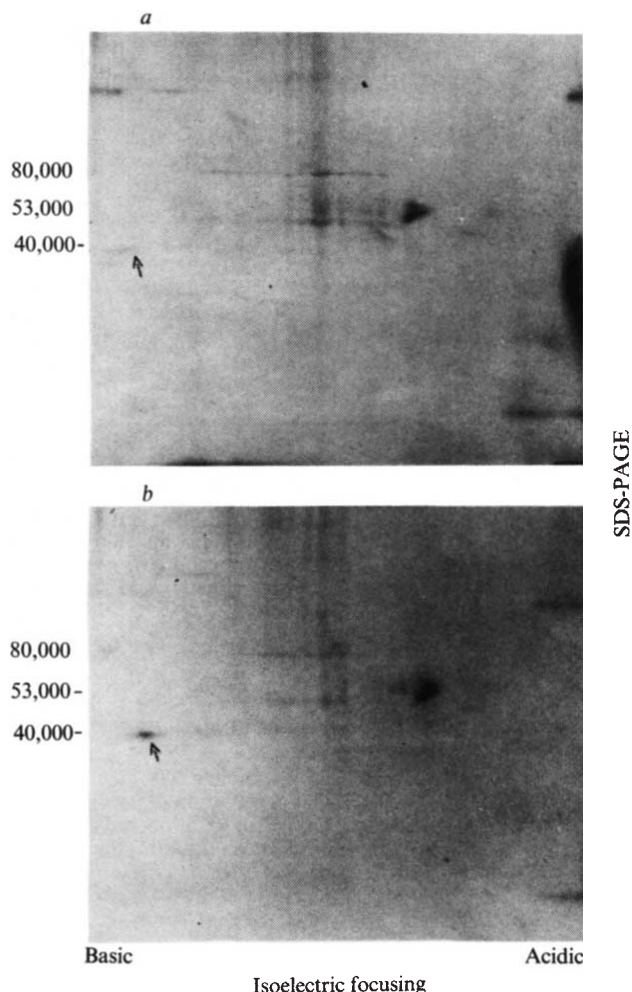


Fig. 3 Autoradiograph demonstrating the distribution in a two-dimensional electrophoretic system¹⁹, of ^{32}P -SPM proteins which were phosphorylated in the absence or presence of 0.01 mg ml^{-1} PBK. The arrows indicate the position of the 40,000-MW material which migrates to a quite basic region in the isoelectric dimension of the gel. Note that in the fraction incubated with PBK there is a large increase, compared to control, in the amount of label incorporated into the 40,000-MW material. The tissue was phosphorylated in conditions described in Fig. 1 and 20 s after introduction of the $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ a $10 \mu\text{l}$ stop solution was added to the incubation to yield final concentrations of 0.1% (w/v) SDS and 1% (v/v) β -mercaptoethanol. The samples were lyophilised, resuspended in sample buffer¹⁹, and subjected to a two-dimensional electrophoresis as described by O'Farrell¹⁹ with the exception that 3% ampholytes (pH 3.5-10.0, LKB) were used throughout.

Mitochondrial calcium uptake and oxygen consumption in cystic fibrosis

CYSTIC FIBROSIS (CF), an autosomal recessive exocrinopathy, is characterised by chronic obstructive lung disease and gastrointestinal malabsorption. Reports of exocrine gland secretory anomalies in CF¹ and increased calcium ion concentrations in some CF secretions²⁻⁵, and the importance of cellular Ca to stimulus-secretion coupling⁶ led us to study cellular Ca in CF. We previously reported an enlarged intracellular Ca pool in skin fibroblasts from subjects with CF and obligate heterozygotes, as measured by ⁴⁵Ca exchange into and out of whole cells⁷. We suggested that the altered Ca pool represented a change in mitochondrial Ca. The finding of such an alteration in cells from obligate heterozygotes, who cannot be detected as possessing the CF gene before the birth of a child with CF, suggests that the Ca finding is related to the basic gene defect in CF. The intracellular site of the altered Ca pool or pools could not be identified by studies with whole cells but required measurement of Ca uptake by isolated cell organelles. Here, we report data on Ca uptake studies with mitochondrial and microsomal fractions isolated from skin fibroblasts of subjects with CF and age-, sex- and passage number-matched controls in which mitochondria from CF cells accumulate more Ca than those from control cells. We also describe subsequent studies which show increased mitochondrial electron transport activity, measured as oxygen uptake by whole cells, in cells from subjects with CF and obligate heterozygotes. All experiments were carried out with cells at passage 4-10.

Cells were grown to confluence in roller bottles as previously described⁷, collected by rubber scraper, washed and pelleted by centrifugation in cold 0.85% NaCl. Homogenisation of the resulting cell pellet (approximately 5 mg protein) was carried out at 4 °C in 5 ml 0.25 M sucrose containing 1 mM EDTA and 10 mM Tris-HCl, pH 7.4. Fifteen strokes with a tight-fitting Dounce homogeniser were sufficient to yield 90% cell disruption as assayed by light microscopy. Three subcellular fractions were recovered by differential centrifugation: unbroken cells and nuclei at 600g for 10 min, mitochondria at 20,000g for 15 min, and microsomes at 105,000g for 45 min. Succinate dehydrogenase (SDH) activity determinations⁸ were carried out on samples of cell homogenates and isolated fractions. SDH activity per mg protein in the mitochondrial fraction was 30-fold that in the cell homogenate and there was no measurable SDH activity in the microsomal fraction. Cell fractionation was carried out simultaneously on CF and control cell lines, and Ca uptake measurements were promptly carried out on the isolated fractions following procedures for mitochondrial⁹ and microsomal¹⁰ fractions.

Mitochondria isolated from normal human skin fibroblasts accumulated Ca (Fig. 1) with a capacity and time course similar to mitochondria from other tissues¹¹. We believe this to be the first report of Ca uptake by mitochondria isolated from a nontransformed human cell type. CF mitochondria accumulated more Ca than control mitochondria (Fig. 1), and Ca uptake in CF mitochondria was about 78% more than in controls at 30 min (nmol Ca per mg mitochondrial protein: CF = 122.7 ± 10.7, control = 69.1 ± 6.0, *n* = 15 pairs). This difference was highly significant (*P* < 0.0005). In contrast to the marked difference in Ca uptake measured between CF and control mitochondria, the microsomal fractions from CF and control cells accumulated equal amounts of Ca (nmol per mg protein measured at 15 min: CF = 23.5 ± 3.1, control = 27.6 ± 3.7, *n* = 8 pairs). These findings indicate an altered mitochondrial capacity for Ca uptake in CF fibroblasts sufficient to account for the previously reported increase in CF whole cell Ca^{7,12}. The microsomal fraction from skin fibroblasts does not contribute to the CF Ca difference observed in these cells.

Ca uptake measured on whole cell homogenates after the method of Ash and Bygrave¹³ further supported the hypothesis of altered mitochondrial uptake in CF fibroblasts. In this experiment Ca uptake was determined in the presence and absence of ruthenium red, a potent and specific inhibitor of mitochondrial Ca uptake, which allowed a breakdown of the total Ca uptake by the cell homogenate into two portions, one attributable to mitochondrial (ruthenium red sensitive) and the other to all extra-mitochondrial Ca accumulation. The 8-min measurement taken after a plateau in Ca uptake was reached revealed a greater total Ca uptake in four CF cell-line homogenates compared with paired control values (110.10 and 93.43 nmol per mg protein, respectively), matched pair Student's *t*-test *P* < 0.01, and a greater mitochondrial Ca uptake in CF cell homogenates compared with paired control values (101.28 and 83.73 nmol per mg protein, respectively), matched pair *t*-test *P* < 0.005. The entire difference in Ca uptake between CF and control cell homogenates is accounted for by the increased Ca accumulated by CF mitochondria.

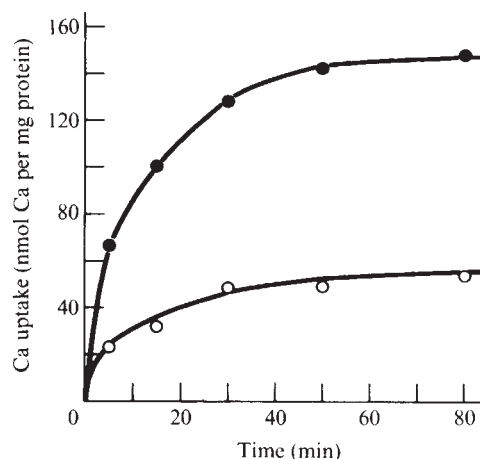


Fig. 1 The time course of Ca uptake per mg mitochondrial protein by CF (●) and control (○) mitochondria. Each point represents the mean of duplicate determinations on two CF and two control mitochondrial preparations. Isolated mitochondrial fractions (~500 µg protein) were washed twice in EDTA-free 0.25 M sucrose/Tris, pH 7.4, then suspended in solution A (Tris-HCl 33.6 µmol, KCl 24 µmol, MgCl₂ 3 µmol, and sucrose 25 µmol per 400 µl, pH 7.4). A 100-µl aliquot of mitochondrial suspension was delivered to each experimental tube (no. 2051 Falcon) containing 400 µl solution A at 20 °C with air as the gas phase. After a 10-min incubation, 100 µl of solution B (sodium succinate 6 µmol, KH₂PO₄ 1.8 µmol, and ATP 0.45 µmol per 100 µl, pH 7.4) was added, and 60 s later 100 nmol ⁴⁵CaCl₂ were added in a 100-µl volume. At each sampling time point a 100-µl aliquot was removed from the buffer and delivered to a microcentrifuge tube (500 µl) containing 100 µl of quench solution (20 mM EGTA and 10 µM ruthenium red). The quenching tubes were centrifuged at 12,000g for 4 min to pellet the microcentrifuge, and a 100-µl sample of the supernatant was taken for ⁴⁵Ca determination.

Mitochondrial Ca uptake and release is vital to Ca homeostasis in many cell types. In such cells, Ca-dependent processes may be fundamentally under the influence of the mitochondrial Ca pool. Release of Ca by mitochondria gives a rapidly available Ca source and sequestration by mitochondria provides a major Ca sink. Cytoplasmic Ca, even in discrete areas of the cell, has been shown to be controlled by local mitochondria¹⁴.

One can hypothesise a mechanism to link the altered mitochondrial Ca in CF fibroblasts and the major signs associated with the disease. Much of the pathology of CF is caused by secretion of highly viscous mucus in the respiratory and gastrointestinal systems¹. Increased viscosity of CF mucus can

be attributed to high levels of Ca in these secretions^{15,16} and Ca in secretory cells is closely associated with secretory vesicles¹⁷. The enlarged intracellular Ca pool present in fibroblasts may also exist in exocrine cells in CF and may account for the increased levels of Ca in some CF secretions. Such an hypothesis demands further substantiation, but it is very significant that altered cell function in CF may be due to altered Ca homeostasis.

Ca uptake by mitochondria is a complex reaction driven by the proton gradient established across the inner mitochondrial membrane by the electron transport system^{18,19}, and this system may form the basis for the observed Ca alteration in CF cells. We have begun to study electron transport activity in CF fibroblasts by measuring oxygen uptake by whole cells.

CF, heterozygote and respective control fibroblasts were collected by trypsin digestion and suspended in Hank's balanced salt solution with 1 mg% bovine serum albumin and 200 mg% dextrose. Oxygen consumption by the suspended cells was measured with an oxygen electrode (model 53, Yellow Springs Instruments) at a cell concentration of approximately 1×10^7 cells per 3 ml buffer. Both CF and heterozygote fibroblasts consumed oxygen at a significantly higher rate than their respective control fibroblasts (n atoms oxygen per min per 10^8 cells: CF = 335.4 ± 28.7 , control = 170.7 ± 28.5 , $n = 5$ pairs, $P < 0.005$; heterozygote = 227.7 ± 17.7 , control = 156.3 ± 15.6 , $n = 8$ pairs, $P < 0.005$). The measured oxygen uptake was completely inhibited by cyanide, indicating that it was mitochondrial. These data suggest that the electron transport system in CF and obligate heterozygote fibroblasts is more active than that in respective controls, in the conditions of this study.

The apparent central role of Ca in several pathogenetic pathways in CF and the data reported here in which both mitochondrial Ca capacity and oxygen consumption are increased in CF suggest that this organelle is a suitable focus for CF investigations.

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Intraspecific hybridisation and the release of mutator activity

It has been suggested by Grant^{1,2} that mutation and hybridisation may not be independent of one another as sources of genetic variation in natural populations. Indeed, it has been shown in both plants and animals³⁻⁸ that hybridisation can stimulate the production of new mutations and chromosome breakage events. The possible role of intraspecific hybridisation in inducing genetic change has recently been discussed in various contexts, especially in relation to the action of mutator genes in natural populations^{1,2,9-11}. We have previously suggested that mutator factors, which are known to induce both visible and lethal mutations and chromosome breakage, are commonly present in natural populations of *Drosophila melanogaster*. Within subpopulations, however, these mutators are genetically suppressed. On hybridisation between subpopulations, genetic suppression breaks down, resulting in the release of mutator activity and, hence, in an explosive increase in genetic variation.

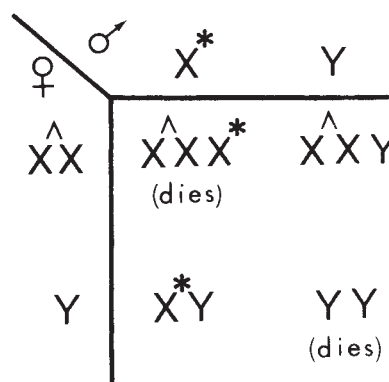


Fig. 1 Progeny from a cross of *D. melanogaster* females with attached-X chromosomes and males from natural population lines or a control line. X* = X chromosomes scored for spontaneous sex-linked visible mutations in male progeny.

Clearly, these explosions of mutation, which have been documented in some natural populations^{12,13}, might have an important role in evolution and speciation. Here, we test this hypothesis of 'hybrid release' directly by determining whether intraspecific hybridisation increases the frequency of mutation above that found in the sampled natural population. Our study differs significantly from other assays of mutator gene effect¹⁴⁻¹⁶ in that mutation frequencies before and after hybridisation are compared directly. The results show that hybrid release of mutator activity does occur as the result of hybridisation, producing a severalfold increase in the frequency of mutation. Thus, hybrid release may be a major mechanism in the induction of genetic variation in natural populations and so may be a driving force in evolution.

Two sets of experiments were carried out, one assaying X-linked visible mutations, the other X-linked recessive lethal mutations. The rationale in both experiments is to determine the frequency of mutation events before and after hybridisation of a wild-type *D. melanogaster* strain with a laboratory strain. The wild-type strains included 'OK1' (which has been observed to undergo recombination in males¹⁷ and frequent chromosome breakage in spermatocytes¹⁸ after outcrossing to laboratory stocks, and is typical of the large number of wild mutator strains that have been collected worldwide⁹), 'GR' males (collected from a Grand Rapids, Ohio, population in June 1978), and 'NO' males (collected from a Noble, Oklahoma, population in July and August 1978). The inbred wild-type laboratory strain Canton-S served as a control. C(1)DX, y f (a marked attached-X stock) and Basc (a marked X chromosome balancer stock)

Table 1 Sex-linked visible and recessive lethal mutation frequencies before and after hybridisation of wild-type strains and a control stock

Males tested	Visible mutants*			Lethal mutants†		
	Mutants	Total	%	Mutants	Total	%
Wild base population	0	13,355	0	31	10,496	0.30
Wild hybrid males	43	16,501	0.26	95	10,288	0.92
Control (Canton-S) base population	0	8,590	0			
Control (Canton-S) hybrid males	0	8,803	0			

Visible mutants were scored using OK1 as the wild population following the breeding programme of Fig. 1; recessive lethal mutations were scored using Grand Rapids males as the wild population and the breeding programme described in the text.

* For wild population $P < 0.001$.

† $P < 0.001$.

were used to manipulate tested chromosomes in the breeding programmes¹⁹.

The experiment to assay X-linked recessive visible mutations is based on the transmission of *Drosophila* attached-X chromosomes (Fig. 1). The only surviving offspring of a cross between females with attached-X chromosomes and wild-type males are attached-X females and males which have received their X chromosome from their father. Thus, in the first generation of such a cross, the phenotypes of male offspring will provide a direct measure of mutation in the homozygous wild-type base population; any mutant male will have been the result of mutation in a sperm formed before hybridisation. These male progeny, however, are hybrids, being heterozygous for each of the autosomes. Mutation events that occur in their developing sperm can be detected by repeating the cross to attached-X females. A comparison of mutation frequency in the second cross (hybrid males) to that in the first cross (base stock males) will show the effect of hybridisation on mutation frequency. Data from these crosses are summarised in Table 1.

No visible mutations were found among 13,355 tested X chromosomes from the base population, but a mutation frequency of 0.26% was found among 16,501 X chromosomes in hybrid males of the second cross. In a smaller sample of chromosomes tested with NO males, no visible mutations were found among 1,726 tested X chromosomes in the first cross, whereas 9 visible mutations were found among 3,483 tested chromosomes in the second cross (0.26%). The absence of visible mutations is apparently typical of first crosses of wild lines. Although a test of second-cross hybrids has not been done with two other wild Oklahoma populations, no visible mutations have been detected in a total of 9,510 tested first-cross chromosomes (0/4,578 from Stratford, Oklahoma and 0/4,932 from Tishomingo, Oklahoma, populations). As a control, the wild-type laboratory strain, Canton-S, which has been observed to be free of mutator factors¹⁸, was also scored for spontaneous sex-linked visible mutations in base stock males and in hybrid males. No increase in mutation frequency was observed after hybridisation (Table 1). The results of this control experiment show that the increase in mutation frequency after hybridisation in the crosses with natural population lines is due to hybrid release of mutator factors in the natural population lines and not in the laboratory stock.

The most common mutations were allelic to the *singed* bristle mutation, although a variety of body and eye colour mutants, rough eye, and bristle size and shape mutations have been found in the hybrid cross. All these mutations bred true in subsequent generations. It is interesting that *singed* was also the most

common mutant type found by Green *et al.*^{20,21} in their studies of mutator genes. In addition, their mutants were unstable, suggesting that they might have been induced by the reversible incorporation of an episome or other factor into the chromosome^{20,21}. No revertants have yet been found in our mutants among only a small sample scored, but one case of a second (*singed*) mutation occurring in a mutant line (*yellow* body colour) demonstrates that mutation induction is not limited to the first hybrid generation.

As the X chromosomes in the first and second crosses are the same, any difference in mutation frequencies is due to the intraspecific hybridisation between lines. There is no doubt that these data show a marked increase in mutation frequency in hybrids. The visible mutations induced in a line, however, are only a small proportion of the total mutations, the largest class being recessive lethals. Thus, we confirmed these observations by assaying recessive lethal mutation frequencies in crosses equivalent to the first and second crosses described above.

Recessive sex-linked lethal mutations were detected by crossing 51 individual wild-caught GR males to Basc females. From each cross, individual GR/Basc females were then mated with Basc males, and their progeny scored for recessive X-linked lethals by noting the presence (no lethal) or absence (lethal) of wild-type males among the progeny. Any lethals would have occurred during the sperm development of the base population wild males, making this cross equivalent to the first cross described for visible mutations. Hybrid GR males from each of the 51 lines were scored by crossing single viable hybrid GR males (heterozygous for the Basc autosomes) to Basc females and repeating the original crosses.

A comparison of recessive lethal mutation frequencies in base population males and hybrid males is shown in Table 1. The hybrid release hypothesis would again predict that the frequency of lethal mutations in the hybrid males would be higher than that in the base population males. This is precisely what is found. A threefold increase in mutation frequency is observed on hybridisation.

The results from these experiments show that intraspecific hybridisation does increase the frequency of mutations, and provides strong evidence in favour of the hypothesis of hybrid release of mutator activity. It has been well established that mutators are present in many *Drosophila* populations⁹ and probably in other organisms^{4,5,22}. The assumption that mutators are genetically suppressed within subpopulations has been supported by these results and by studies of chromosome breakage in *Drosophila* spermatocytes¹⁸ which were analysed directly in the base population.

Indeed, the genetic suppression of mutator activity within a subpopulation is a consequence that is readily predictable from the action of natural selection^{6,10,11}. However, one would not necessarily expect the same genes to be involved in the suppression of mutation in different subpopulations. Thus, hybridisation would lead to heterozygosity of suppressor genes and to further breakdown by segregation in later generations. This hypothesis makes several predictions, such as the involvement of recessive rather than dominant suppressor loci, which are testable in principle. The increase in mutation frequency and chromosome breakage events observed after hybridisation could also be part of a larger syndrome of genetic and developmental changes that occur with the disruption of adapted gene complexes. Further work in this area should contribute to a better understanding of the role of mutator genes in generating the variability that has a major role in the response of populations to changing environments and to competition from other populations.

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Gene numbers as measured by single-copy DNA saturation with mRNA are routinely overestimates

A MAJOR experimental approach to the elucidation of the role of differential gene expression in developmental processes has been to measure the number of different mRNA sequences in eukaryotic cells¹. This number has been obtained from the percentage of radioactive single-copy DNA rendered double-stranded during hybridisation with homologous mRNA. The percentage, appropriately corrected for the average size of mRNA and the chemical complexity of the unique DNA of the organisms, can be related to the total number of different genes expressed in the functional mRNAs of the cell type or tissue studied². The number and distribution of different mRNA sequences can also be determined by hybridising radioactive cDNA with excess unlabelled mRNA and comparing its kinetics of hybridisation with a suitable kinetic standard³. However, here, I discuss the use of single-copy DNA saturation with mRNA, and suggest that gene numbers obtained in this way are routinely overestimates.

Although both the above methods have disadvantages, especially because of difficulties in driving the reactions to completion, quite consistent measurements have been obtained for some organisms^{4,5}. However, gene numbers as determined by the two experimental approaches often differ appreciably. As shown in Table 1, it is striking that cDNA values obtained even in the most favourable conditions⁶ are generally less than those of single-copy DNA measurements. This may be due to the experimental shortcomings of the cDNA method as suggested by Hereford and Rosbash⁷.

However, we should consider the basic assumptions underlying interpretation of single-copy saturation data². Most single-copy sequences were presumed to be of about the same length as mRNA and to be flanked by repetitive sequences. Single-copy sequences, isolated from randomly sheared nuclear DNA by

repeated fractionation at C_0t levels at which most repetitive sequences should have reassociated, were thought to be a representative probe of total unique DNA. Furthermore, it was thought that coding sequences were rendered double-stranded by complementary RNA sequences over their whole length due to the short tracer length (200–300 nucleotides) relative to mRNA length. Both assumptions, if invalid, would create a bias towards too high gene numbers.

Eukaryotic genomes contain much repetitive DNA⁸, most of which is intimately interspersed with unique DNA⁹. It has been shown that the reassociation analysis of sheared nuclear DNA does not reveal all the unique DNA because of its interspersed with sequence classes with faster kinetics¹⁰. The cryptic unique DNA content of a genome may be estimated by further experiments which allow the extrapolation of reassociation kinetics to zero tracer fragment length. In the first such experiment, gene number estimation was explicitly based on the total genomic unique DNA content. This rationale has also been used in other investigations^{11,12}. ³H-labelled single-copy DNA used for such saturation experiments will, however, be enriched for the fraction of single-copy DNA that is organised in long, continuous stretches. Thus, cryptic single-copy sequences will seldom, if ever, be present in such preparations, whereas sequences from long stretches of unique DNA will be almost exclusively non-cryptic and will be over-represented by a factor depending on the ratio of total unique DNA to the kinetically resolved unique DNA. This is because, from an individual unique sequence of length, L_u , using hydroxyapatite (HAP) assay in reassociation kinetics analysis of total randomly sheared DNA of median length L_m , only the fraction $(L_u - L_m + L_h)/L_u$ will seem to be unique, where L_h is the minimum length of double-stranded DNA able to bind to HAP. If L_h is 40 nucleotides and L_m 300 nucleotides, then unique sequences of L_u below 260 nucleotides will not be seen as unique, and sequences of $L_u = 500$ nucleotides will seem to be 50% unique. At a median length of 1,400 nucleotides about 80% of all sequences will seem to be unique. As the mean length of mRNAs is about 1,500 nucleotides and as almost all mRNAs are coded for by unique sequences^{1,11,13}, it is reasonable to assume that most mRNAs will be complementary to unique sequences which mainly behave non-cryptically during reassociation analysis of moderately long sheared DNA (300–400 nucleotides).

If the proposed assumption is valid, gene numbers in higher animals would need to be reduced by a factor of 0.6–1.0. However, gene numbers in plants would be more seriously affected. Higher plant genomes contain a large proportion of repetitive DNA, often amounting to more than 60% (ref. 14). Repetitive DNA sequences occur at short intervals throughout the majority of all unique DNA^{15–19}, thus concealing a large fraction of unique DNA during reassociation analysis. Murray *et al.*¹⁹ have demonstrated that the majority of all unique DNA sequences in the pea are only about 300 nucleotides long. This must also be true of parsley, where we have shown that in the reassociation analysis of 300-nucleotide total nuclear DNA, only 40% of all unique DNA was revealed.

Goldberg *et al.*¹¹ have reported the first measurements of the sequence complexity of polysomal RNA from cells of a higher plant (tobacco leaves). Here, although ³H-labelled single-copy saturation experiments indicated the presence of 27,000 different average-sized mRNAs, cDNA–poly(A)–mRNA hybridisation only revealed the presence of 12,000 different species. This and similar results of our own experiments on higher plants are listed in Table 1.

The strikingly different results obtained by the two methods suggest that the assumptions outlined above may be correct, particularly for plant gene number measurements. It may therefore be necessary to reduce single-copy DNA gene number estimates to take into account the degree of over-representation of mRNA coding sequences in the tracer single-copy DNA preparation. I propose that gene number estimates should be based on the fraction of unique DNA resolvable in reassociation kinetics of 400-nucleotide DNA.

Table 1 Comparison of gene number measurements in various species to show overestimation

Organism	Haploid genome size (nucleotide pairs)	% of scDNA (300 nucleotides) reacting with RNA	% of DNA (300–450 nucleotides) observably unique	% of DNA revealed unique in inter-spersion analysis	% of DNA assumed unique in complexity estimate	Gene number estimate based on assumed unique DNA	Gene number estimate based on observed unique DNA	Gene number estimate based on cDNA hybridisation kinetics
Yeast ⁷	1.3×10^7	20.0	95		95	4,000 (1,500)*	4,000	3,000
Achlya ^{5,24,25}	4.2×10^7	3.76–7.0	82		64	1,850–3,400 (1,150)	2,400–4,400	3,200
Sea urchin								
Embryo ²	8.1×10^8	1.35	50	75	75	14,000 (1,200)	9,300	
Embryo ²⁶ + adult	8.1×10^8	3.0	50	75	75	30,000 (1,200)	20,000	
Chicken								
oviduct	1.23×10^9	1.8	70		70	15,700 (1,900)	15,700	14,000
Tobacco								
leaves	1.65×10^9	2.6	23	42	42	27,000 (1,240)	15,000	12,000
Mouse								
L cells	2.7×10^9	1.3	70		70	26,000 (1,000)	26,000	10,000
Parsley								
Leaves	1.9×10^9	2.54	12	30	30	25,000 (1,400)	10,000	9,200
Callus ^{13,18}	1.9×10^9	3.43	12	30	30	35,000 (1,400)	13,700	11,500
Rat								
⁶	2.9×10^9	1.43	70		70	31,000 (1,800)	31,000	23,000
Barley								
^{30,31}								
leaves	6.1×10^9	1.46	12	25	25	30,000 (1,500)	14,400	

* Figures in parentheses are nucleotides.

In view of recent findings on gene structure^{20,21}, the assumption that during hybridisation, coding sequences are rendered double-stranded over their whole length, may also be incorrect. The organisation of eukaryotic genes may be based on the pattern of the ovalbumin gene²². Here, quite short coding pieces alternate with longer spacers of noncoding DNA belonging to the same kinetic DNA class, that is, unique DNA. However, this would mean that random shearing of genomic DNA and hybridisation with homologous mRNA would enlarge the fraction of single-copy DNA bound to HAP during the standard assay procedure. Hybridised coding sequences will bear additional single-copy DNA single-strand tails, thus apparently representing a larger coding sequence complexity than they really comprise.

Consider a coding element of DNA (an exon) of length L_e sheared to a median length, L_m , and separated from other coding sequences by spacers of length L_s . Then, this stretch of DNA after saturation hybridisation to RNA and analysis by HAP chromatography, may form hybrids over a length of $L = L_e + 2(L_m - L_h)$ and statistically will seem to be hybridised over a length of

$$L = L_e + L_m - 2L_h \quad \text{if } L_m - L_h < L_s$$

and

$$L = L_e + L_s - 2L_h \quad \text{if } L_m - L_h > L_s$$

L/L_i will give an estimate of the overhybridisation. For an arbitrary case of an intron 200 nucleotides long, with fragments of mean length 250 nucleotides, a space of length 400 nucleotides, and HAP binding to a minimum length of 40 nucleotides of double-stranded nucleic acid, the overestimation of the length of the intron would be 1.86-fold.

A way of avoiding such overestimates would be to use the S_1 nuclease assay instead of the HAP assay. A procedure adapted for experiments where low background values are of crucial importance has been developed by Maxwell *et al.*²³

If both the above considerations are broadly correct, the new scDNA-based gene numbers would be less than the corresponding cDNA numbers. However, it seems that the two assumptions will not be wholly applicable. There will be at least some small unique DNA genes and also some genes with

intervening repetitive DNA spacer sequences, as suggested by the finding of repetitive sequences in animal hnRNA¹.

However, taking into account these modified assumptions, it seems possible to reconcile results of gene number measurements obtained by different methods. This is particularly so for differences in plant experiments. The results reinterpreted in this way will reduce gene number estimates for plants from some 30,000 to 10–15,000 different active genes in cells and tissues examined.

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matters arising

Holocene reef growth on the edge of the Florida Shelf

LIGHTY *ET AL.*¹ have raised several important lines of speculation concerning the growth of reefs in the Florida and Bahamas areas and elsewhere in the Caribbean. The principal contribution of these authors has been to show an effective growth of a shallow-water coral-frame assemblage dominated by *Acropora palmata* in a period between $9,440 \pm 85$ and $7,145 \pm 80$ yr BP. This extends the evidence previously presented by Macintyre and Glynn², Adey³ and Easton and Olson⁴ for progressive growth over the past 6,000 yr.

The structures described lie within a depth range (15–30 m) which, only 40 km to the south, is accessible to Recent coral growth. One might speculate that, where conditions of growth are more favourable, reefs of the same age as those described by Lighty *et al.*¹ have been buried beneath growth covering the period from ~7,000 yr BP to the present.

Observations by Goreau and Goreau⁵ and Goreau and Land⁶ in Jamaica and by Newall and Rigby⁷ and Shinn⁸ and myself in the Florida–Bahamas province show a remarkable similarity of offshore profiles. The first element is a gentle slope to ~10–15 m depth (visible as a distinctive terrace on Fig. 1 of Lighty *et al.*¹). It is from this that the major reefs in Florida (for example, Carysfort, Dry Rocks, Alligator Reef, and so on) rise and, at least on Andros and Eleuthera, so do the major reef patches. Seawards, the surface slopes more steeply to about 30 m before plunging almost vertically in “the wall” described by Ginsburg and James⁹, Goreau and Land⁶ and others. The illustrations of Goreau and Goreau⁵ seem to apply throughout the Caribbean area and suggest only two shelf-edge growth sites. Figure 1 of Lighty *et al.*¹ suggests a third, from 15 m.

We need to exercise some care in interpreting terrace levels as much of the mobile sand is derived from the excess production of the shallow-water zone (see Neumann and Land¹⁰ and Moore *et al.*¹¹). However, the widespread occurrence within these offshore platforms of exposed Pleistocene limestones indicates that we are not dealing with accretion levels but with surfaces prepared by erosion during glacial periods (compare Purdy¹²).

Now, although there are no readily

available figures for growth rates at 30 m depth there is certainly subjective evidence that they are slow. There are commonly <2 m of ‘frame’ projecting above the sand surface. In effect, relief from present-day surfaces in these depths is no greater, and may perhaps be less, than on the ‘inactive’ structures to the north.

Thus, it seems reasonable to assume first that surfaces which could have supported active coral growth at a time of rising sea level did not and, second, that the structures which we now see at 30 m are in effect the surfaces of frames which were first formed 7,000–10,000 yr ago. There is an obvious incentive to test them, using the methods described by Macintyre¹³, bearing in mind the suggestion of Macintyre¹⁴ that structures on terraces at 30–80 m depth in the eastern Caribbean do indeed represent an older phase of accretion.

We may now rephrase the question posed by Lighty *et al.*¹ What event, taking place ~7,000 yr ago, inhibited all shallow-water reef growth in the Caribbean? This growth was never able to resume in their northern area, presumably for the reasons which they accept pertaining to cold surface waters. In southern areas, however, growth began on new sites along the edge of the inner break in slope, forming the present shelf edge patches.

Lighty *et al.*¹ provide figures to show that an *Acropora palmata* reef is capable of keeping pace with rising sea level. There seems little reason to doubt this capability, but did it in fact do so? I believe that it did not and that, far from creeping upwards along the surface of a submerging slope, the principal locus of growth was established on a new and much shallower site. We are left with the conclusion that although sea level rose throughout the Caribbean there was a relatively abrupt change at ~7,000 yr BP. The broad outlines of reef morphology may have been defined at some earlier date but it was this event which determined the areas in which corals would grow. Sadly, in the area described by Lighty *et al.*¹ growth was no longer possible.

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LIGHTY *ET AL.* REPLY—The points raised by Braithwaite have been discussed in several studies of Caribbean Holocene reefs. As mentioned in our letter¹, for example, core data from a relict reef (depth 18 m) off southeastern St Croix indicated that a flourishing, shallow-water *Acropora palmata* (Lamarck) barrier reef was killed about 8,000 to 9,000 yr BP, when the shelf was flooded². Increased water turbidity related to the erosion of soil cover was thought to be responsible for the demise of this early Holocene reef. By the time water conditions returned to normal, the depth of water was too great for an *A. palmata* reef to be re-established. Subsequently, the substrate was colonised by a slowly accreting, deeper water community consisting of coral heads and octocorals.

That study² and others^{3,4} have proposed that turbidity, related to shelf erosion accompanying rising sea level, has generally prevented the continuous accumulation of Holocene reefs on wide shelf platforms of the Caribbean that are deeper than 10 m below present sea level. Although impressive reef structures are present on platforms <10 m below present sea level, these structures cannot be older than 4,000–7,000 yr BP, which is the period of initial flooding of these platforms. In south Florida, for example, the present reef tract has developed over the past 7,000 yr (ref. 5) on shallow areas of the shelf, behind the relict, shelf-edge reef of early Holocene age⁶.

Some deeper shelf areas isolated from the effects of shelf erosion might have continuous Holocene reef sections dating back to 10,000 yr BP, but no such section has yet been found.

Note, however, that in protected areas,

the fragile and rapidly growing *Acropora cervicornis* (Lamarck) is capable of constructing extensive reef structures in depths of 10–15 m (ref. 7). A Holocene reef section 33.5 m thick that consists primarily of this coral has been drilled off the lee side of the Alacran reef complex^{7,8}.

Evidence thus far indicates that Holocene reef history in the Western Atlantic is governed by factors related to shelf depth, extent of Pleistocene sediment cover on these shelves before flooding, degree of protection allowing the accumulation of *Acropora cervicornis* in intermediate water depths, and water temperatures in areas near the northerly limits of present-day reef development.

There is no evidence to indicate that a major 'event' 7,000 yr ago inhibited reef growth in the Western Atlantic. The death of shelf-edge reefs in this region is related to the time of shelf flooding, which varies from area to area, depending on local bathymetry. Some reefs (such as Galeta Reef⁹ and South Florida reef⁵) were, in fact, established on shallow-shelf areas around 7,000 yr BP. Another point to clarify is that relict reefs died when the rate of rising sea level was decreasing, rather than increasing, so that their death cannot be related to their inability to keep pace with the rising seas.

Braithwaite implies that reef growth, if uninterrupted, would accumulate by "creeping upwards along the surface of a submerging slope". Evidence suggests, however, that the relict, early Holocene reefs, in response to relatively rapid changes in sea level, favoured vertical over lateral growth. Reef accretion is therefore limited to linear trends that form narrow ridges; these ridges are a dominant feature of the sea floor topography of outer shelves and upper slopes in the eastern Caribbean¹⁰.

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Matters Arising

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Climatic signals in tree-ring chronologies for British oaks

HUGHES *et al.*¹ claim that analyses of the ring-widths of British oaks should make it 'possible to produce maps of annual or even seasonal climatic anomalies' and that these 'would provide a significantly more detailed picture of climate in the past 250 years than is now available'. This claim is based on what they regard as clear climatic signals from regression analysis applied to monthly means of temperature and precipitation and to mean ring-widths of 10–35 oaks for periods of 44–60 yr on three sites around the Irish Sea.

In fact, as their Fig. 1 shows, few of the responses to the various monthly means have 95% confidence limits that are significantly different from zero, and they are also inconsistent from one site to another. Thus the effect of temperature is positive in April and negative in May at Rostrevor but the reverse at Raehills. They do not mention any test for reproducibility on a site either by using different sets of trees or successive periods of time.

Our criticism of the work does not relate to the statistical method with its use of principal components² that Hughes *et al.* use: this has proved successful where the growth of trees is primarily controlled by one climatic factor. We believe the weakness of the claim comes from two limitations.

The first is the unsuitability of the data used. Chronologies of mean ring-widths were developed for dating by dendrochronology and are not necessarily appropriate to dendroclimatology. Monthly meteorological means are merely convenient summaries of climate: their use with oak is suspect because in different years the date of bud-burst varies by several weeks and therefore frost damage to young leaves can apply in a different calendar month. Other climatic anomalies and extremes are also obscured by the use of monthly means.

When regression analysis is used in an exploratory role, the result will be reliable

only if the major factors which affect the tree growth are included. The second limitation concerns the failure to determine the extent to which the oaks suffered damage from defoliating caterpillars, and to include this factor, if appreciable, in the analysis. Measurements of the considerable reduction of the annual growth of oak as a result of damage by caterpillars and other insects have been made in Russia³, Germany⁴, Denmark, (K. Christensen, unpublished data), and by ourselves at Oxford⁵, and warnings of the implications of this factor on dendroclimatology expressed. In the light of these considerations, the exploratory work reported by Hughes *et al.* requires evidence for reproducibility before their claim to have found clear climatic responses can be accepted. Our studies on oaks show that monthly correlations for the past 45 yr of growth with monthly climatic data are not maintained when the past 90 yr are examined.

One successful application in Europe of the Fritts method has been the outcome of preliminary studies to select a suitable parameter for use in the analysis in conjunction with a climatic stress that is strong, reproducible and not vitiated by the limitations of monthly meteorological data. Schweingruber, Bräker and Schär⁶ found that damage by insects, such as the larch-bud moth on conifers in the Alps, could be avoided by using the maximum annual density (derived by X-ray radiography) in place of ring-widths. In this way, the growth of alpine conifers on many sites in the Alps were found to be consistently related to the summer temperatures in July, August and September. We believe that the future of dendroclimatology in the British Isles needs a similar experimental approach to achieve success.

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HUGHES ET AL. REPLY—Varley *et al.*'s comments are based largely on misunderstandings of dendroclimatological techniques²⁻⁷. We agree that it is not appropriate to use mean ring-width chronologies. We did not do so but rather used standardised indices of ring-width² for well-documented reasons^{5,7,8}. We did not use regression analysis of ring-widths on monthly meteorological figures as suggested¹. We stated clearly² that ring-width indices were regressed on the eigenvectors of climatic data. Consequently our results should not be compared with those referred to by Varley *et al.*^{1,9} where no account has been taken of the non-orthogonality of the data. We have maximised tree-ring variance attributable to the eigenvectors of climate, not the number of significant response function elements. The two are not simply related⁵. We discussed differences between the reported response functions. Such 'inconsistency' was ecologically expected and dendroclimatologically welcome^{5,10}. The choice of meteorological variables related to climatic reconstruction, not tree physiology⁵.

In their reference to defoliators Varley *et al.* miss the fundamental point of dendroclimatology. The material selected for site chronologies conforms strongly to a common pattern of ring-width variation². This could only be produced by factors acting similarly and synchronously on all the trees selected. No sound evidence for caterpillars doing this on British oaks is produced¹. The European examples^{11,12} concern aggregate effects on stand samples, not dendrochronologically selected material. The English example^{9,13} rests on a regression analysis of % mean latewood increment and % mean caterpillar numbers (5 trees, 20 yr) where no test of difference of the gradient from zero is given. As Varley¹³ uses the intercept to estimate effect of caterpillars it is surprising that no confidence limits are given. Incomplete statistics are given for an ecologically incomplete analysis¹⁴.

Our results are inevitably reproducible within sites as only strongly cross-dated trees are used. Chronologies from subsets of 13 and 10 trees (S. J. Milsom, unpublished data) at Maentwrog are extremely similar ($t = 15.4$, 261 yr overlap)¹⁵ as is the Rostrevor chronology¹⁶ and one from 11 nearby trees ($t = 17.4$, 223 yr overlap). Consequently they produce very similar response functions except for two differences in precipitation elements between the Rostrevor sites.

We will not use response functions to reconstruct climate. For this, we will use transfer functions between time-series of spatial anomalies of tree-rings and climatic variables³⁻⁵. Recent work (B.G., unpublished data) has shown there to be spatially coherent, temporally consistent patterns of ring-width variability. Very similar patterns for the first five eigenvectors accounted for 65% (1825-94) and

70% (1895-1964) of variance in a 15-chronology tree-ring grid between 50-60° N and 10° W-10° E. Patterns of UK mean monthly temperature (B. Gray, unpublished data) and annual rainfall (R. Tabony, unpublished data) share similar properties. It is such coherent, consistent large-scale patterns of tree-ring variation that will be calibrated for use in reconstructing past climate. These new results further support our expectation that tree-ring chronologies from the British Isles will provide valuable proxy climatic records² and bring the generation of testable reconstructions closer.

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Cell density is determined by a diffusion-limited process

FROM the culture of 3T3 cells in media with various viscosities, Whittenberger and Glaser¹ have concluded that the saturation density of these cells is not dependent on a diffusion-limited factor. A flaw exists in the design of their experiments. In their culture conditions the cell

Table 1 Effect of viscosity on cell density

Treatment	Cell density ($\times 10^{-4}$ cm ⁻²)
1% Serum	9.5 $\pm$ 1.7
1% Serum plus 1% Methocel	3.8 $\pm$ 0.6
1% Serum plus 1.5% Methocel	2.5 $\pm$ 0.7
1% Serum plus 2% Methocel	1.3 $\pm$ 1.1

Swiss 3T3 (clone 4A) cells were plated on 14-mm glass coverslips in Dulbecco modified Eagle's medium containing 10% serum. After 1 d single coverslips, with cells at an initial density of 3×10^3 cells cm⁻², were transferred, one coverslip each, to 15-cm plastic culture dishes. To each dish 50 ml of medium was added with or without methylcellulose (Methocel A4M, Dow) as indicated above. Treatments were in triplicate. After a further 5 d incubation in a CO₂ incubator, the coverslips were removed, washed gently with Tris-saline buffer, and the cells were fixed in formal-saline. The cells were stained and 10 fields per coverslip were counted under a microscope with an eyepiece grid. (Means and s.d. are given for the 30 fields counted per treatment.) Observations under the microscope indicated that there was no loss of cells during the washing and fixing procedures. The cell densities at 5 d approach equilibrium densities². Approximately the same variation of cell density with viscosity was observed when the coverslips were placed individually in 100-ml glass beakers containing 50 ml of medium with the same Methocel concentrations listed above, indicating that the geometry of the culture vessel is not critical. The low cell density observed in medium with 2% Methocel was also found when the cells, on a coverslip, were beneath a 3-mm layer of viscous medium (2% Methocel) in the bottom of a beaker, and 50 ml of medium without Methocel was placed above the viscous layer. Therefore, it is not the absence of convection currents in the bulk of the medium that leads to the lower cell density. The Methocel used was shown to be non-toxic by culturing the cells in a small volume of medium (0.2 ml cm⁻² of cells) with 1% serum, with and without Methocel, in normal culture conditions. As was observed by Whittenberger and Glaser¹, Methocel had little effect on the cell density attained. As Dextran and Ficoll give lower than normal cell densities at 5 d in normal culture conditions¹ (attributed by the authors to 'toxicity' of these agents), these materials were not investigated.

density of 3T3 cells is limited primarily by the total supply of serum growth factors in the medium, not simply by the concentration of serum. As high viscosity does not alter the total supply of medium constituents, it is not to be expected that the cell density attained should be affected by viscosity in their conditions.

In the culture conditions that were used, with the usual small volume of medium (0.2 ml cm⁻² of cells), quiescent 3T3 cells destroy serum growth factors quickly after each medium change and before the cells have been exposed long enough for many cells to initiate DNA synthesis. Any increase in cell number after a medium change is balanced by cell loss when the concentrations of serum growth factors reach their minimum levels before the next medium change. High viscosity, if it has any effect, will prolong the period of destruction of serum factors in these conditions, and this prolongation will lengthen the exposure of the cells to a serum concentration high enough to initiate DNA synthesis. This might even increase the cell density. Therefore, the experiments of Whittenberger and Glaser

are inconclusive. Fortunately, there is a simple approach that avoids these problems.

This approach involves culturing 3T3 cells with a sufficiently large volume of medium (20 ml cm⁻² of cells) to maintain effectively a steady-state concentration of serum. Culturing 3T3 cells on a small coverslip in a large volume of medium² results in much higher cell densities and demonstrates the importance of the total supply of serum factors. For example, with a large volume of medium containing 1% serum, 3T3 cells grow to a 'saturation density' expected in 10% serum, that is, to approximately 10 times the cell density attained in normal culture conditions with a small volume of medium². Therefore, if the supply of medium is adequate, a 1% serum concentration will support the growth of high density 3T3 cells. A conclusion from the 1971 experiments, still not generally appreciated, is that the saturation density of 3T3 cells observed in normal culture conditions in 10% serum is not the cell density that is in equilibrium with 10% serum; the cell density in equilibrium with 10% serum is much higher². Instead, the saturation density observed with 10% serum is the cell density that is in equilibrium with the depleted level of serum growth factors present in conditioned medium. This is the cell density that is re-established in a 'wound'. The earlier experiments make clear that, with 3T3 cells, equilibrium conditions require a large volume of medium relative to the cell area.

As high viscosity can lower the effective local concentrations of diffusible factors in the medium but not their total supply, a meaningful test of the effect of high viscosity on cell density must be carried out in conditions that provide a large excess of medium. We have carried out such an experiment and the results are summarised in Table 1. The cell density is clearly affected by the viscosity of the medium. In the absence of methylcellulose, a high cell density is attained. As the viscosity is increased, the cell density decreases. These results demonstrate that the saturation density of 3T3 cells is in fact determined by a diffusion-limited process when the culture conditions are appropriate to test the importance of diffusion.

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WHITTENBERGER AND GLASER
REPLY—Holley and Baldwin disagree with our conclusion¹ that the growth of 3T3 cells in 10% serum is not limited by the rate of diffusion of mitogens to the cell surface. They have carried out experiments similar to ours in 1% serum in a large volume of medium and they observe an effect of Methocel on the cell directly after 5 d of growth. Thus in their conditions they conclude that growth is limited by diffusion and then extrapolate from this to our previous experiments.

While the lack of an effect of a viscous polymer on growth indicates that viscosity has no effect on growth in the specified conditions, the observation of such an effect is not unequivocal, as it is possible to consider that at low serum concentrations the polymer (in this case Methocel) actually binds mitogenic factors. Also we do not know whether the results of Holley and Baldwin represent a change in the rate of growth or in the limiting cell density. In addition, we need to consider the possibility that as the concentration of serum components is decreased a new situation arises in which the rate or extent of growth does become diffusion limited.

In 1971 Holley and Kiernan² reported that incubation of confluent 3T3 cells with growth medium containing 10% serum depleted the medium of mitogenic factors, although the data were not presented. We believe that Table 1 represents a confirmation of their original data. This experiment shows that incubation of medium with confluent cells for 24 or 72 h reduced their mitogenic effect on 'sparse' growth-arrested cells by about 40%, which, because of the shape of the saturation curve, is equivalent to that of 1.5% serum. Thus serum must contain a rapidly utilised mitogen(s) and more stable mitogens. However, these experiments suggest that daily addition of fresh medium to confluent 3T3 cells should result in continued growth (at 60% of maximum) unless at high density other factors become limiting. This is essentially the conclusion of the Dulbecco wounding experiments³. The two factors that have been proposed are an effect of cell contact or a limitation by diffusion. Our experiments seem to rule out diffusion and therefore support the notion that cell to cell contact is important in this phenomenon. This is not meant to imply that in different conditions the rate of growth may not be influenced by the rate of mitogen diffusion to cells. Previous experiments by Thrash and Cunningham also seem to rule out mitogen depletion as the primary reason that cells cease to grow at high density⁵.

The implication by Holley and Baldwin that when diffusion is limited the cells are exposed to mitogen for longer periods of time is correct, but as the steady state concentration of mitogen that they see is lower, it seems unlikely that this will result in an increased cell density. While there is

Table 1 Ability of confluent 3T3 cells to deplete medium of mitogenic factors

Medium	DNA Synthesis	
	Rate per 4 h (relative amount)	% Labelled nuclei (0-24 h)
0.2%	1	0.7
2%	19	69
5%	24	71
10%	24	72
Depleted media		
24 h	14	48
72 h	16	44

Swiss 3T3 cells were plated in 2 cm² Linbro multi-well trays at 1.5×10^5 cells ml⁻¹, 1.0 ml per well, in Dulbecco modified Eagle's medium (DMEM) containing 10% calf serum. Cells to be pulsed always received L-³⁵S-methionine to 1 μ Ci ml⁻¹ to label cellular protein. After 72 h plating the medium was changed to DMEM containing 0.2% serum in 0.6 ml per well in order to render the cells quiescent. One day later the medium was changed to DMEM containing 0.2% dialysed calf serum. Three days later the medium was changed again to DMEM containing either 0.2%, 2.0%, 5.0%, or 10% dialysed calf serum (0.6 ml per well). For chronic labelling ³H-thymidine was also added to 1 μ Ci ml⁻¹, 6.7 Ci mmol⁻¹. Some dishes received medium from confluent cultures (in 35-mm dishes, 2.0 ml per 8 cm²) of 3T3 cells. This medium had been added to the cells either 24 or 72 h previously as DMEM per 10% dialysed calf serum. At the time of removal of the medium from the monolayers the cells had DNA synthesis rates of 0.6% (72-h₃ medium) or 12.8% (24-h medium) as determined by a 2-h pulse with ³H-thymidine and compared with the rate of sparse cultures. Twenty hours after the last medium change some wells were pulsed with ³H-thymidine (3 μ Ci ml⁻¹, 6.7 Ci mmol⁻¹) for 4 h and processed as described.

some loss of cells at high density this cannot be greater than the total rate of cell division of confluent cells which is generally less than 5% that of sparse cells.

Thus while we believe that Methocel affects the growth of sparse cells in 1% serum in the conditions described by Holley and Baldwin, we do not believe that this observation affects our original conclusion. If growth were limited by diffusion of mitogens in our conditions, we would have observed an effect both on the rate of growth and on the final cell density, which we did not observe.

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reviews

Szilard and his bomb

Edward Bullard

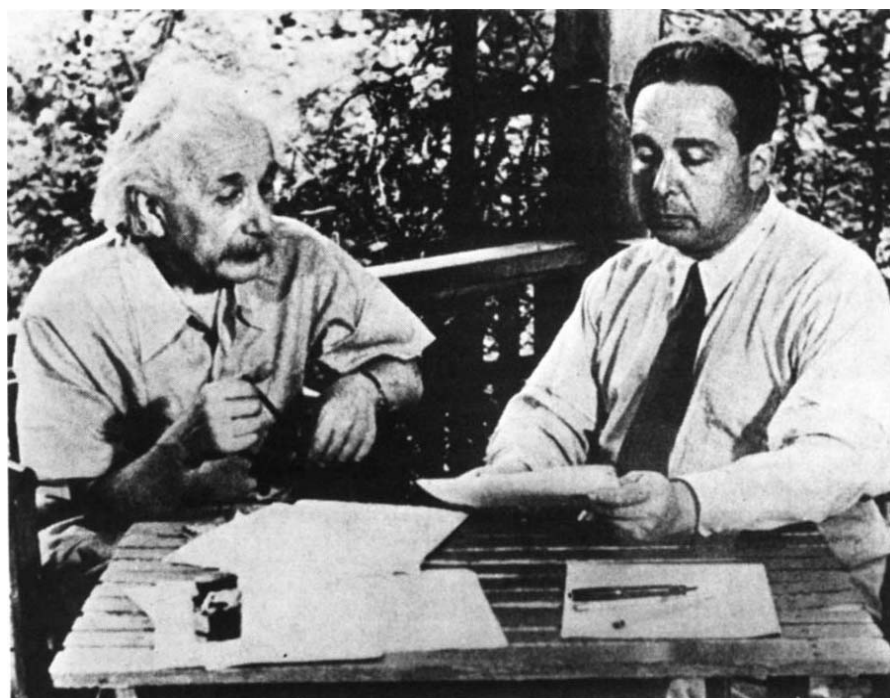
Leo Szilard: His Version of the Facts. Selected Letters and Correspondence. Edited by S. R. Weart and Gertrud Weiss. Szilard. Pp.244. (MIT Press: Cambridge, Massachusetts, and London, 1978.) \$17.50; £12.25.

SZILARD'S scientific and technical papers were published in 1972 as Vol. 1 of his *Collected Works*. The present volume deals with political and organisational questions concerning the development of nuclear weapons before and during World War II.

Unfortunately Szilard did not write an autobiography; he did, however, record his thoughts on tape and preserved copies of letters and memoranda. The book is compiled from this material. It consists of seven chapters arranged chronologically; each has a few pages of introductory narrative skilfully patched together from Szilard's tapes and notes; and these are followed by related letters and memoranda.

The first chapter gives an account of Szilard's life from his birth in Budapest in 1898 to the end of 1938. It includes a plan by the 32 year old Szilard for a very elitist organisation intended to train a group of intelligent young men who were to form and guide public and official opinion. With hindsight one can easily see such a body turning into the Hitler Jugend or the Young Communist League. It is a little surprising that anyone as far sighted as Szilard should have taken the scheme seriously. How far sighted he was is clear from his remark, made at the age of 16, that he did not know how World War I would end but he knew that it ought to end by the defeat of Germany and Russia. By the end of chapter 1 he has passed through Berlin, London and Oxford, has taken out patents on a nuclear reactor and bomb and moved to New York.

Szilard's patents of 1934 presupposed the existence of a reaction in which the impact of a neutron on a nucleus released more than one neutron from the target. No such reaction was known, but in 1939 Hahn discovered neutron-induced fission. Szilard at once saw that neutrons would be emitted from the fragments and that there was a good chance of his reactor and his bomb becoming a reality.



The rest of the book has three themes; first, his efforts to show that a reactor and a bomb are possible; second, his attempts to restrict the spread of the knowledge of the possibility; and third, his efforts to persuade the government that nuclear bombs should not be used on Japanese cities.

The first part of the programme was entirely successful. In August 1939 Szilard persuaded Einstein to write to President Roosevelt saying that it seemed likely that a nuclear bomb could be made. This was the start of interest by the US Government in the matter. The illustration shows a reenactment of the meeting between Szilard and Einstein (no photograph was taken at the time.) A little later Szilard and Fermi invented the graphite-moderated uranium reactor which was in operation in Chicago at the end of 1942. Similar reactors at Hanford produced the plutonium used in the bomb dropped on Nagasaki. The second part was clearly hopeless; after the discovery of fission the possibility of a chain reaction was bound to occur, and did occur, to many people. The use of the bomb was also inevitable, though it is greatly to the credit of Szilard and those who supported him that they did

attempt to prevent it. Use of the bombs was inevitable in the face of the gloomy prognostications of the casualties likely to occur in the landings in Japan, which were to take place early in November 1945.

Szilard was one of the group of Hungarian emigrés who made such a deep impression on American science, particularly on theoretical physics in the 1930s and 1940s. On the whole they got on well together (Szilard tells Einstein that Edward Teller is 'besonders nett' and has letters from Wigner signed 'Wigwam'). Relationships with the management of the Chicago laboratory and of the Manhattan project were something quite different. Szilard seems to have had little confidence in any of those concerned. In fact, arguments about Szilard's patents became so acrimonious that in October, 1942, two months before his reactor first went critical, Arthur Compton, the Director of the Chicago laboratory, ordered Szilard to leave (he later relented). Relations would have been even worse had Szilard known that General Groves had drafted an order for his internment; this information has only very recently been unearthed (see Bernstein, B. G., *Inquiry*, 8 January, 1979, p27).

Why did things go so badly for Szi-

lard? Was it just that he felt he had a mission to save the world? Was it that he and his friends were almost all foreigners? Was it perhaps that the Chicago lab had no charismatic leader, such as Oppenheimer was at Los Alamos. Oppenheimer seems to have been able to cope with General Groves and his entourage even when doing things much more open to misconstruction than anything Szilard ever did.

The book is 'Szilard's version of the facts' and of events that have had an overwhelming influence on the relationships of science and government

and on the position of science in the modern world. It is not the whole picture, there are all kinds of cross links and loose ends that need to be traced out. It is, however, a book that must be read by anyone interested in World War II or in the roots of our present scientific establishment. It is also a story about the impact of America on a very clever and deeply humane man, coming in a time of crisis from a quite different tradition. □

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Infeld's sketches

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Why I Left Canada: Reflections on Science and Politics. By Leopold Infeld. Pp. 212. (McGill-Queen's University Press: Montreal and London, 1978.) \$16.95.

LEOPOLD INFELD was internationally renowned for his important contributions to theoretical physics, as well as for his gift to write on science for the layman. But he was also an extraordinary personality and had an unusually interesting life. He was a legend in his native Poland even in the pre-War years. I happened to do my stint of teaching in the grammar school in Warsaw where Infeld had been teaching twelve years earlier, and his exploits, mainly of a romantic nature, were still a major topic of conversation in the common room. It was many years later that I met him and got to know him.

Infeld described the first 42 years of his life in a remarkable autobiography *The Quest*, published in 1941; the book concluded with his decision to settle in Canada. But nine years later he returned to Poland for good, and there, in 1964, he continued his autobiography in a book entitled *Sketches from the Past*, which consisted of a number of essays. It is these essays, together with one written just before his death in January, 1968, that constitute the book *Why I left Canada*, published in Canada.

The long interval between the writing and publication in English has not detracted from its value. Although I had read *The Sketches from the Past* soon after their publication in Poland, I was still fascinated on re-reading them in English. The perfect translation (by Infeld's widow) has succeeded

in retaining the characteristic flavour, and the introduction and notes added to the English edition have enhanced the book, although in my opinion it would be more appreciated if it were read together with *The Quest*.

The title of the book *Why I left Canada* is taken from the title of one of the essays. In it Infeld describes the 'atom spy' hysteria in Canada in the post-War years, and which resulted in

those who obstructed his academic career in pre-War Poland for anti-semitic reasons. But above all his decision must have been influenced by the terrible devastation of Polish science brought about by the War.

The events after his return are described in the essay 'Poland'. Infeld did not suffer from modesty, but the essay only hints at the great heights he reached when he came back to Poland. To the public he was a hero, and officialdom treated him with reverence. As far as science was concerned his word was command, and he used his prestige and influence to foster the reconstruction of science in Poland. Naturally, his own subject did best, and in a very short time he set up the Institute of Theoretical Physics, with fifty scientists working in it.

Not all was sugar and spice. Although he was in sympathy with the regime, he was too much of an independent character to accept its rigidity. In particular, he was galled by the idolatry of Stalin, which was normal practice in Polish science in the early 1950s. At one time he even re-



Einstein and Infeld

the hounding of Infeld and his family after he had made a short visit to Poland in 1949. He was planning to spend the following year in Poland on a sabbatical, but when his Canadian passport was withdrawn he decided to remain in Poland for the rest of his life. The Canadian Government revenged itself in the subsequent cancellation of Canadian citizenship of his two children, who were born in Canada.

Although the harassment in Canada was the direct reason for Infeld's return to Poland, it is very likely that he would have done it in any case. There were several reasons for this. Politically, he had always been on the left (he considered himself a Marxist); the new regime in Poland therefore appealed to him much more than the 'flesh pots' of Canadian life. There may also have been an element of personal satisfaction in getting the upper hand over

gretted having returned to Poland. But a few years after Stalin's death the atmosphere became more relaxed, and later he was even able to write about it in the book published in communist Poland.

The essay 'Einstein' describes the contacts with him in the last years of Einstein's life. Infeld had a very successful collaboration with Einstein in the 1930s, and he resumed it after the War, first directly, when Infeld was still in Canada, and later by correspondence. Although Infeld did not agree with Einstein about the unified field theory, they worked in harmony, as is evident from the exchange of letters. Some of Einstein's letters were published in the book for the first time in English, and this is particularly welcome in the year of Einstein's centenary.

Throughout the book Infeld portrays many scientists, living and dead, whom

he met, and in general he speaks about them with kindness. But he has no kind word for Oppenheimer, in an essay written in the last year of Infeld's life and published after his death. The cause of his wrath was a lecture on Einstein given by Oppenheimer in 1965, and which—in the version available to Infeld—was an amazing denigration of Einstein. Point by point, Infeld proved Oppenheimer's distortions, and in the process he lashed out

at him with all the might of his pen. Even the news of Oppenheimer's death, which reached Infeld while he was writing the essay, did not mollify him, because he believed that the saying that one should speak only good of the dead does not apply when referring to a figure belonging to history.

I adhered to this view in writing this review of Infeld's book. □

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Journey through relativity

Joe Schwartz

Einstein's Universe. Written by Nigel Calder. Narrated by Peter Ustinov. Produced by Martin Freeth. (BBC-TV: London; WGBH: Boston, 1979.) First UK transmission: 14 March, 1979, BBC-2, 1900 h. Repeated 18 March.

I HAD watched one of Nigel Calder's previous BBC science extravaganzas—*Key to the Universe*—with friends: an immunologist, a developmental biologist and a psychotherapist. The developmental biologist went upstairs, the psychotherapist fell asleep (we didn't wake her), and the immunologist and I discussed immunology. Calder's approach to physics is characterised by a "gosh, isn't science grand" school-boy attitude and in *Key to the Universe*, his taste could be given free rein. Quarks (with colour and flavour), strangeness, hypercharge, charm, were right up his alley. Calder is captivated by the terminology of high energy physics and obviously enjoys the mystery and incomprehensibility of it all. This view is not uncommon among science journalists. James Gorman, a New York writer, confessed last year: "I value physics, not for how well it explains the world, but much as the irreligious loved the Latin mass, for how it sounds."

So I waited for *Einstein's Universe* with some apprehension. Einstein hated obfuscation and mystification. "Since the mathematicians have attacked relativity, I myself no longer understand it", he wrote sarcastically in 1909. The core of his work has long been acclaimed for its simplicity. Special relativity (1905) is based on two simple physical propositions; all steady motion is relative and there are no instantaneous interactions in nature.

General relativity (1916) is built on the simple physical fact that all objects fall at the same rate in a gravitational field.

Einstein is legendary for having been able to extract far-reaching conclusions from these slim physical clues. In the special theory he replaced Newton's metaphysical absolutes of space and time with a material absolute; there are no instantaneous interactions in nature, no signal can propagate faster than the speed of light. In the general theory, Einstein put geometry on a material basis. The validity of Euclidean geometry became a problem for physical investigation, not for abstract contemplation. Experiment would decide whether we live in a Euclidean world where two parallel lines meet at infinity or whether we do not. (We do not.)

Calder's programme was far better than I expected. *Einstein's Universe* is a major improvement over *Key to the Universe* with numerous innovations that need to be developed further. The use of Peter Ustinov as a foil avoids the phenomenon of authoritative talking heads speaking directly to the camera. Moving the set out of the studio and into a live observatory (McDonald) gives more immediacy. The models and animations are quite effective, particularly the curved billiard table. Roger Penrose projects an intensity and seriousness that is a welcome contrast to the usual bland pronouncements from the scientific orthodoxy. And the informal discussion at the end of the programme expressed some of the tone and quality of research work at the frontiers of physical theory.

But it is not enough. I will be greatly surprised if many viewers found their interest sustained for the entire show. The problem lies in the script. Calder concludes the show with an Einstein quote: "The most beautiful thing that we can experience is the mysterious." But Einstein, for whom clarity was paramount, did not mean mystery at the hands of his fellow human beings. He meant the concrete mysteries of nature. The mystery the programme leaves us is a lack of

physical understanding of the astounding results of relativity; black holes, the expanding universe, clocks running slow, $E=mc^2$. We do not know how the theory produces these results or what were the problems for which these results are the solutions. It is Einstein turned on his head: the results of the investigations are seen to be mysterious, not the problems that gave rise to them in the first place.

Wheeler's summary of general relativity is excellent. "Space tells matter how to move and matter tells space how to curve." But how many people will be able to say that they understand what Wheeler means. It is irritating to hear the constancy of the speed of light, the physical basis of the special theory, repeatedly referred to as Einstein's "obsession". The physics has got lost in the whizzing shots of complex machinery, cables, flashing lights and intricate experimental arrangements. If the point is to explain and not to mystify, a way has to be found to make the equipment more than a mere spectacle. Einstein actually was quite knowledgeable about experimental work. In 1908 he and a friend Paul Habicht patented a design for a sensitive voltmeter accurate to half a millivolt which was in advance of the instruments of the time.

The striking results of the theory get

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us in the front door. But once we are there nothing much is shown. Where are the workers in the field, the graduate students, the post-docs. Where are the arguments. Some experimentalists and theorists do not believe in black holes. Where are they. The Ustinov character is an advance but he is too passive. We need a character who can force the explainer to acknowledge that some of the ideas are poorly understood and insufficiently tested. We should see some actual data instead of simply being told that an experiment satisfies Einstein's theory to 1%. The human substance is missing and the presentation of shocks and thrills wears thin half-way through.

Journalists have to dig harder for their stories. They need to use the same

critical sense they reserve for other subjects and apply it to science reporting. A more critical less idolised approach is needed. Einstein recognised this problem himself: "In my view, there is but one way to bring a great scientist to the attention of a larger public; it is to discuss and explain the *problems* and solutions which have characterised his life work. Otherwise the result is a banal hero-worship based on emotions and not on insight." [Emphasis added.] Science is not more objective and mysterious than any other human social activity. Everyone in science knows this. It is time to start showing it to the public. □

Joe Schwartz is a freelance journalist on leave from the City University of New York.

Foundations of relativity

P. C. W. Davies

General Relativity from A to B. By Robert Geroch. Pp.225. (University of Chicago Press: Chicago and London, 1978.) £8.40; \$11.95.

TEN years ago introductory books on general relativity were a rarity. Since then, advances in the subject and a greater awareness among students that gravity is an intriguing, even bizarre, branch of physics, have stimulated a stream of works appealing to the bottom end of the market. An enormous number of people want an appetite-wetter that will "get them into" gravity without the traditional years of investment in advanced mathematics, and not only so that they can bore dinner guests with virtuoso expositions about black holes. Often more formal treatments do not succeed in communicating the sense of awe and iconoclasm that stems from topics such as total gravitational collapse, event horizons and singularities in spacetime. In addition, mathematically based texts tend to ignore the reader's struggle for sound physical intuition, and depend upon a sort of intellectual osmosis in which feeling for the physics is supposed to slowly and spontaneously filter through the formulae.

Robert Geroch is a man of outstanding international reputation, whose personal contributions to the subject of general relativity are great. He is also a skilful expositor, and has taken enormous trouble to write a book in which the reader starts out from basics. The book is a product of a lecture course given to non-science under-

graduates at the University of Chicago, but it can be read with profit by science specialists who want to acquire a historical and philosophical perspective, to scrutinise the fundamental concepts that underpin the theory of relativity, and to perceive what is a rather specialised branch of physics, with a human face. The text is liberally punctuated by line drawings, some of which might, but should not, frighten the potential reader browsing through the pages.

Part A of the book dwells, perhaps a little too laboriously, on the image of our world as a spacetime threaded by worldlines of matter. To communicate the crucial and fundamental concept of the relativity of motion, much space is devoted to the old and discredited Aristotelian and Galilean concepts of space and time. These sections certainly help to focus the reader's attention on the basic issues at stake, but carry the danger of making Aristotle seem more reasonable than Einstein. There is a sense in which relativity is equally as elegant and natural as the everyday, intuitive view of space and time, but a historical approach to the subject does not always do it justice.

The greater part of the book (Part B) gets to grips with general relativity in its full splendour. Professor Geroch adroitly guides the reader through the geometrical basis for gravity without burdening him with elaborate formalism or needless jargon. Every new concept is strongly rooted in concrete physics. Meter sticks, clocks, light signals, records—people—inhabit the author's models, observing and measuring all sorts of phenomena. Great emphasis is laid on physical meaning and consistency, on asking and answering exactly what is meant by distance, interval, and so forth. It is quite the most elaborate discussion of the funda-

mental aspects of relativity that I have encountered in a non-specialist book.

The price paid for this admirable but extended devotion to "getting it straight" is that Einstein's actual theory does not surface until three-quarters of the way through the book, and when it does, perhaps inevitably for a book at this level, it is largely in the form of analogy. This leaves very little room for applying the theory to anything exciting, but a mandatory chapter on black holes is included. Readers who have taken the effort to get this far will find that the weird and wonderful properties of these entities can in fact be understood in a straightforward way now that the spacetime geometry concept has been so thoroughly well explained. Professor Geroch explicitly excuses himself from



Einstein expounding to Ramsay MacDonald

what he calls "gee-whizzes", but still manages to convey the immensely engaging nature of the black holes subject. I was a little disappointed not to see an extensive discussion of singularities, and also the big bang and cosmology, but there is always the possibility of a *C to D* sequel.

In summary, *General Relativity A to B* is one of the few books that treats the foundations of the theory of relativity seriously and in elaborate detail, at the non-specialist level. It cannot be read, however, without effort and commitment. The index is rather sparse and the price a shade too high, but otherwise it is a nicely produced book that in spite of its narrow scope illuminates many grey areas of spacetime physics. □

P. C. W. Davies is Lecturer in the Department of Mathematics at King's College, University of London, UK.

obituary

Margaret Mead, 1901–1978

MARGARET MEAD died on 15 November 1978 just a month before her 77th birthday. Thus ended a unique career. Boundlessly energetic and phenomenally productive (her bibliography runs to nearly 4,000 items), alert to every new development in the humane sciences, sensitive to urgent social issues and with a talent for working with and through others, she achieved a world-wide fame.

Her place in the history of American anthropology is assured; for she played a decisive part in its transformation from a narrow academic specialism into a modern humane science with world-wide interests. But she will also have a place in the social history of the United States for her catalytic influence on the development of educational and moral ideals and values after World War II. Field work, she often proclaimed, was the 'living stuff' of anthropology and she made over 20 field trips between 1925 and 1975. But in between spells of writing, teaching and organising research, she also travelled indefatigably in America and abroad, to lecture, to preside at conferences, to advise academic bodies or governmental agencies, and so on. But whatever the occasion might be, she always took her stand as an anthropologist, drawing attention to the many alternatives mankind had invented for dealing with the common problems of personal and social life.

Margaret Mead was born on 16 December 1901 in Philadelphia. As her father was a Professor of Economics and her mother an early student of sociology, she could say with pride, in her autobiography *Blackberry Winter* (1972), that she grew up in a 'social science' family. To their influence was added that of her grandmother, whose example inspired the ideals which made Dr Mead essentially an educator.

Dr Mead came into anthropology by chance. After a miserable year at De Pauw University she moved to Barnard College in New York in 1920. While completing her degree in psychology she was drawn into Franz Boas's circle. What most influenced her, however, was her friendship with Ruth Benedict, Boas's assistant, which was to last a lifetime of close collaboration. Anthropology was in a ferment just then.



Functionalism, with its emphasis on fieldwork and the analysis of the internal integration of primitive societies, had been launched in England. In America the complementary 'configurationist' movement, later to come to brilliant fruition in Benedict's *Patterns of Culture* (1934) was under way. And in the background loomed the figures of Freud, Jung and later Piaget. Mead took something from each to form her guiding principle that culture shapes character, is learned and is lived by individuals.

Mead's fieldwork began in 1925 with her study of a group of adolescent girls in Samoa. She found that unlike American girls, they suffered no emotional crises in the transition to adulthood, and she attributed this to the benign sexual mores and family system of Samoans. Addressing herself to the general public in her deliberately 'novelistic' best-seller *Coming of Age in Samoa* (1928) she drew the moral. A new image emerged of anthropology as a science of public enlightenment. Later, reacting to straight-laced anthropological criticism, in a conventional monograph, and journal articles, she presented the technical anthropological and psychological data on which she based her book; and she did likewise with all her subsequent fieldwork.

Dr Mead's next field study, shared

with her second husband Reo Fortune (her first was the archaeologist Luther Cressman) took her to the lagoon-dwelling Manus of the Admiralty Islands, New Guinea. Here, supplementing ethnographic observation with ingenious psychological tests, she found that contrary to some psychologists' theories children were not naturally animistic. A puritanical, competitive culture made fearful animistic adults of them. Her second popular book, *Growing Up in New Guinea* (1931) was another best-seller.

In 1931, after a depressing interlude in a disintegrating Omaha community (described in Mead's *The Changing Culture of an Indian Tribe*, 1931) they returned to New Guinea. Three strikingly different tribes were visited. There they were joined by Gregory Bateson, fresh from his Iatmul field work. Discussions with him crystallised an hypothesis that cultural patterns incorporated different psychologically identified temperamental types. Mead's *Sex and Temperament in Three Primitive Societies* (1935) developed this hypothesis to explain the startling contrasts in the norms of sexual identity and behaviour in these three tribes. The book, like its predecessors, was severely criticised by some anthropologists. But her argument that what are now called 'gender' differences are culturally inculcated not biologically determined, brilliantly anticipated the position vigorously maintained by women anthropologists today.

Mead elaborated this theme in lectures and articles in both popular and learned journals. Fourteen years later she refashioned it in *Male and Female* (1949), her most influential book. Comparing observations in seven non-Western cultures with the American scene, she examined the cultural variations in the roles of men and women in the reproductive process and in the socialisation of children, and related this to the way the oedipal conflict is resolved, thus drawing attention to the significance of the father in character formation. Here again she anticipated what has become a topic of major research activity among psychologists, anthropologists and some behaviour biologists.

Breaking with Fortune in 1936, she

married Bateson; and this partnership led to the field study in Bali (1936–1939) which they claimed, with some justice, to have been a major advance in anthropology.

In Bali they were faced with a complex oriental civilisation very different from primitive New Guinea, though, as before, their concern was primarily with culture-learning, the ways as they put it 'living persons embody their culture'. Their main innovation was the huge and minutely detailed photographic documentation that foreshadowed the post-war rise to prominence of the ethnographic film. Their fieldwork concentrated on interpersonal relationships and interactions, particularly of parents and children in the context of child-rearing and socialisation processes that were central to Dr Mead's interests. Her imaginative psychological commentary on the photographic record in their book *Balinese Character* (1942) and in other publications, provoked controversy but also stimulated new approaches in ethnological psychology. The 'anthropology of the body' is a new field of research concerned with problems of the cultural specification of postures, gestures, and body images and processes, and it owes much to the pioneering work of Bateson and Mead.

World War II brought unexpected challenges to American anthropologists. Dr Mead worked with various governmental agencies concerned with the war effort. But most important was the leading part she played in the international team of specialists assembled by Ruth Benedict to compile reports on the 'national character' of the belligerents. Field work being ruled out, they resorted to studying 'culture at a distance', through interviews with immigrants and analysis of literary, film and archival sources. As the publications of the team showed—notably Benedict's celebrated study of the Japanese, *The Chrysanthemum and the Sword* (1946) and Mead's passionate morale-booster *And keep your Powder Dry* (1943)—the method was remarkably effective, so much so that the project was continued until 1951 (with 120 participants!) under the auspices of Columbia University.

The war and especially, by Mead's own account, the shock of Hiroshima, brought home to her the urgent need for a new social and moral vision in western, especially American society. The war had broken down the cultural isolationism which she had helped to expose in her popular books. It had also, she learned, produced unprecedented changes in New Guinea. This led her, in 1953, to return to Manus, accompanied by a student couple.

She found the community completely transformed. Partly owing to the war and to Australian government agencies, but mainly to the charismatic leadership of one local man, the people had abandoned their entire traditional way of life and were rapidly adopting western institutions and ideals. Examining these changes in her *New Lives for Old* (1956) Dr Mead concluded that a unanimous primitive community, once convinced of the desirability of western cultural ideals and institutions, can, given inspired leadership, radically change its way of life in a generation. Thenceforth she advocated the wholehearted support by anthropologists of speedy westernisation in primitive communities with the right leadership and aspirations. She visited the Manus thrice again thus keeping benevolently critical track of their progress in self government, in family life, and in personality development, until 1975.

Watching Manus inspired in Dr Mead an abiding concern with the problems of changing society. Thus orientated, between 1953 and 1975 she also visited the other groups in which she had earlier worked, as well as the growing urban centres in New Guinea. Preoccupied now with the problems of reconciling continuity and change in cultural development (as in her book *Continuities in Cultural Evolution*, 1964) she travelled widely in America, the Caribbean, Europe and the Southern Hemisphere, often enough to participate in academic or policy-making activities, and so to enlarge her understanding of urgent world issues. The key to a more enlightened and humane future, she believed, lies in the way the culturally specified relations of successive generations of grandparents, parents and children, where character is shaped and culture is transmitted, are managed. She stressed in particular the models of the alternatives anthropology has revealed for handling the universal problems of intergenerational conflict.

Never hiding her light under a bushel, Margaret Mead yet disdained pretensions to originality. On the contrary she constantly stressed her indebtedness to others, friends, famous scholars, students and predecessors. But as the long list of visiting professorships, honorary degrees, other academic distinctions, and awards and prizes conferred on her testifies, the pre-eminence of her scientific achievements, her devotion to America and her dedication to the ideals of human betterment were widely esteemed. A phenomenon like Margaret Mead could perhaps not have emerged in any other country than modern America. From her base in the American Museum of

Natural History, where she worked, and taught from the time of her appointment as an assistant curator in 1925 to the end of her days, she exercised a decisive influence both on the development of the social and human sciences in America and elsewhere, and on American educational, and moral values and cultural ideals. But she will undoubtedly be best remembered, as she would herself have wished, as one of the most creative anthropologists of this century.

Meyer Fortes

Bernard Halpern

BERNARD N. HALPERN, one of the first workers on antihistamines, died in Paris on 23 September 1978, after suffering for several years from an incurable and painful illness.

Born in 1904 in Russia, Bernard Halpern came to France after some very difficult childhood years, to complete his advanced studies for a degree of Doctor of Medicine (1936). In order to study, he worked for several years as a technical assistant in the physiological laboratory at the Ecole pratique des hautes Etudes, under the direction of J. Gautrelet.

In 1937, Halpern took over as director of the pharmacodynamic research laboratory of the Société Rhône-Poulenc. He left this position in 1945 to join, as a research worker, the Centre National de la Recherche Scientifique and to direct the Pasteur-Vallery-Radot laboratory at the Broussais hospital. In 1949, he was nominated Director of Research at the CNRS; ten years later, he added to these functions those of Director of Laboratories at the Ecole pratique des hautes Etudes. In 1961, Halpern was awarded the Chair of Experimental Medicine at the College de France, a chair formerly held with distinction by Claude Bernard, and was elected a member of the Académie des Sciences in 1964.

After early work with J. Gautrelet on the action of snake venoms, Halpern devoted himself from 1942 onwards to the study of synthetic compounds behaving as antihistamines, at the Rhône-Poulenc laboratories.

This research topic was, it must be said, initiated some years earlier (1937) by G. Ungar, J.-L. Parrot, and D. Bovet, who used in their experiments compounds belonging to the sympathomimetic and sympatholytic groups obtained from E. Fourneau's laboratory at the Institut Pasteur. In the same year (1937), D. Bovet and A. M.

Staub had detected the same antihistamine properties in thymoxyethyl-diethylamine (929 F) and, some years later, the study of some derivatives of phenylethylenediamine and aminopyridine showed equally favourable properties. As a result of other work by A. M. Staub (1939), Halpern proved in 1942 the relatively high antihistamine effect of N-(2-dimethyl aminoethyl)-N-benzylaniline or Antergan on spasms in unstripped muscles and pointed out the possible use of this compound in human therapy.

In 1944, another compound appeared, Neoantergan, equally suitable for human application which, while chemically similar to Antergan, was more active (Bovet *et al.*, 1944). Still more important appeared to be the N-alkylamine derivatives of phenothiazine, among which Halpern noted the efficacy of Phenergan (1946). It is well known that, apart from its antihistamine effects, this compound was found by doctors to be a psychodepressant and formed the starting point for many neuroleptic derivatives currently used in psychiatry.

From 1950, following research by Jancsó, the work of Halpern and his collaborators took another direction: the functional study of the reticulo-endothelial system (RES). Profiting from the granulopoietic activity of this tissue, they investigated the removal from the blood of various colloids as a function of time. These data once established, the authors found that stimulation of the RES increases the level of immunity of the organism against various infections.

In 1959, Bernard Halpern showed, for the first time, that administration of BCG increases the resistance of the organism to malignant tumors.

In 1964, he described the outstanding immuno-stimulatory properties of *Corynebacterium parvum* on the reticulo-endothelial system. This effect is shown by the increase in the phagocytic index of macrophages and by the increase in liver volume and of splenic cells. Later, Halpern established that the administration of *Corynebacterium parvum* inhibits the development of grafted malignant tumours and their propensity to metastasis. In May 1974, he chaired the first international conference on *Corynebacterium parvum* and its use in oncology, a conference which has led us to revise our ideas of the relations between the host and the malignant tumour. *Corynebacterium parvum* has been employed for several years in human therapy in the treatment of cancers in combination with antimitotic chemotherapy and the results of this method appear promising.

G. Valette

W. O. James

PROFESSOR WILLIAM OWEN JAMES, FRS, who died on 15 September 1978 will be remembered as one of the leading British plant physiologists of this century.

The major part of his academic life as a scholar, teacher and author (from 1929 to 1958) was spent as Demonstrator and then Reader in the Department of Botany at Oxford University. He was elected to the Royal Society in 1952. Latterly from 1958 until his retirement in 1967 he was an innovative Head of Botany at Imperial College, London at a time when the college was rapidly expanding.

During his professional life of over 50 years, W. O. James made many distinguished contributions to several different fields of plant physiology including photosynthesis, mineral nutrition, anaerobic and aerobic respiratory metabolism, alkaloid metabolism and to the understanding of the function of subcellular organelles. His endeavour was always to discover and understand the biochemical events responsible for the physiological functions of plants and to account for these responses quantitatively. He wrote with exceptional clarity and his many scientific papers and several books reflect his scholarly analysis of plant physiological phenomena and his imaginative application of contemporary experimental techniques as aids in understanding them. He retained an enthusiasm for plant biology to the end of his life and was always particularly delighted to hear of progress in the several areas of research in which he himself had been involved.

W. O. James brought to the study of the many-faceted problems of the physiology of plants incisive thought and great care in the design and execution of experiments. These qualities were already manifest in his first paper on the external factors affecting photosynthesis reporting his studies as a graduate student under F. F. Blackmann in Cambridge in the mid 1920s. James demonstrated departures from the limiting factor form of the curve for photosynthesis and explained why they were to be expected. During his postdoctoral years, spent from 1925 in V. H. Blackmann's unit of Plant Physiology at Rothamsted, his interest in mineral nutrition of plants, particularly of potassium, developed, and on his move to Oxford in 1927, the effects of potassium deficit on extractable enzyme activity and respiration rate were among the first problems he tackled. He soon realised that useful interpretation of the experimental effects of mineral nutrition on plants was severely limited by lack of under-

standing of the respiratory processes occurring in normal plants and he and his associates turned their attention to the study of plant respiration.

It is for his contributions to our understanding of plant respiration that James will probably be best remembered. His group in Oxford carried out some of the first definitive work on glycolytic systems in plants and in the early 1940s demonstrated unequivocally that pyruvate was produced from fructose diphosphate in extracts of barley. Later they examined the enzyme systems involved using the phosphorylated intermediates and cofactors which had recently become available commercially, and showed the key enzyme enolase was present in barley sap.

His group published detailed descriptions of the respiratory characteristics of several different plant tissues including leaves, roots and embryos and sought to explain their *in vivo* respiration quantitatively in terms of the activity of enzymes which could be isolated from them. It was demonstrated, for example, that sugar lost from carrot tissue during a period in N₂ could be accounted for entirely by alcohol and CO₂ production. In attempts to explain the terminal oxidation processes in plants whose respiration was differentially inhibited by specific respiratory inhibitors, James and his associates also devoted considerable attention to the examination of soluble and insoluble plant oxidases. Much useful detailed information about the distribution and activity of the variety of plant oxidases was assembled, although no convincing evidence that any oxidase other than cytochrome oxidase plays a major role in plant respiration has yet emerged. H. Beevers in James' laboratory in the late 1940s first examined the exceedingly rapid cyanide-insensitive respiration of the *Arum spadix* and later the Oxford group were the first to show the location of this activity in isolated mitochondria. James' years of study of the respiratory processes in plants culminated in the publication of his book *Plant Respiration* in 1953, hailed as a scholarly summary of a whole epoch of plant respiratory studies.

During his years in Oxford, in addition to his research activities, James also taught plant physiology to several generations of undergraduates and, during the second World War, was a prime mover in the Oxford Medicinal Plants Scheme. The objective of the scheme was to supply medicinal drugs by extracting them from natural plant sources. Many of these drugs were alkaloids and in addition to improving methods for their extraction and

analysis, his group obtained much fundamental information about the synthesis and internal concentration of alkaloids in cells.

Shortly before his move to London, James' group had been amongst the first in the world to isolate active mitochondria from plants and study their oxidative and phosphorylative capacities. The group had also begun to use the new techniques becoming available for the examination of the ultrastructure of cell organelles for James had the vision to see the potential of structural studies in understanding intracellular metabolic processes. At Imperial College he set up a Unit of Analytical Cytology where electron microscopists and biochemists worked together to isolate, purify and characterise subcellular plant organelles. New electron microscopical techniques were developed to monitor the purity and integrity of the isolated organelles used in a continuation of the studies of mitochondria to localise cellular respiratory activities. Later plant nuclei, ribosomes and chloroplasts were examined and the group were the first to publish methods for the isolation of structurally intact chloroplasts and also plastoglobuli and grana from them.

In addition to introducing the study and teaching of analytical cytology of plant cells, James also initiated and fostered the development of new sections of the department at Imperial College to study plant taxonomy, palaeobotany and nitrogen fixation. He guided and encouraged those who shared his enthusiasm for all aspects of plant biology and by example and positive criticism showed the high standard of scientific expertise required to gain a true insight into plant function.

In addition to publishing numerous papers reporting his own research findings, for many years James was a co-editor of *New Phytologist* and a member of the editorial panel of *Endeavour*. His uncluttered literary style also contributed to his skill as a good writer of school and university texts and semi-popular articles and books. All his publications were characterised by the high standard of the beautifully presented and appropriate illustrations. For almost 50 years his now classical text *Introduction to Plant Physiology* (first edition 1931, seventh edition 1972) has been the introduction for successive generations of schoolchildren and undergraduates to this field. In the preface to the fourth edition he looks forward to the time when the reader of his book will take part in finding some of the answers to unsolved physiological problems: indeed there have been

many whose initial interest was aroused by his text and who have gone on to make their own contributions as professional plant physiologists. His *Introduction to Plant Biology* is still used as 'the bible' for school and university courses. His amusing book *Background to Gardening* became a best-seller. After his retirement he published *Cell Respiration* (1971) which gives a comprehensive account of the respiration of the whole range of living cells.

James' distinguished contributions as a scientist were made despite recurrent ill-health throughout his life. All who knew W. O. James and his wife recognise the magnificent supportive role she played throughout his career. His many former colleagues now working in all parts of the world have cause to be grateful to W. O. James for his scholarly example.

R. M. Leech

Francis Aylward

PROFESSOR FRANCIS AYLWARD who was Professor of Food Science in the University of Reading from 1968 to 1976 died on 28 September 1978, aged 67. A memorial service for him was held in Westminster Cathedral on Thursday, 7 December.

After graduating at Liverpool University with first class honours in chemistry, he remained at Liverpool studying lipid chemistry under Hilditch (whose school was of international repute) and obtained his PhD. His subsequent contribution to lipid chemistry and biochemistry was recognised by the award of a DSc in 1958.

Francis began his scientific career by holding research fellowships at the Hannah Dairy Research Institute and Johns Hopkins University. The war years were spent with ICI Ltd. In 1947 he was appointed Head of the Department of Chemistry and Food Technology at the Borough Polytechnic. It was at this time that it began to be recognised, largely as a result of food research carried out in USA and UK, that a substantial and systematic body of knowledge had accumulated, and the time had arrived for food technology to be taught as a discipline particularly to provide the food industry (which was rapidly expanding and moving from a craft based to a scientifically oriented industry) with its scientific manpower. The Borough Polytechnic was one such teaching institution that recognised this need, and under Francis's able and progressive leadership the food technology element of his department grew and flourished.

Though still retaining his interest in lipids he began to take a greater interest in the role of food technology in relation to nutrition and food supplies.

This led naturally to his appointment in 1960 to serve for two years as FAO Adviser on Food and Nutrition to the Government of Ghana, and for the period 1961-62 as Head of the newly created Department of Biochemistry, Nutrition and Food Science at the University of Ghana. From that time he developed a major interest in nutrition in relation to national and international food problems. From Ghana he moved to Poland and during 1962-65 he was Director of the Polish Food and Nutrition Project supported by the UN special fund.

In 1965 he returned to Britain as Director of the Fruit and Vegetable Preservation Research Association, Chipping Campden. Then in 1968, following the retirement of Professor Crossley as Professor of Dairying and the decision of Reading University to broaden the interests of the department to cover food science, Francis was appointed its first professor. In many ways this was an apt and fortunate appointment. Francis believed that institutes of higher education have a duty to assist developing countries, and this view agreed well with the tradition of Reading's Faculty of Agriculture. He obtained a substantial grant from the Leverhulme Foundation which permitted him to support two Senior Fellows and with their help he built up a large postgraduate school of food science—the largest in Europe—drawing most of its students from developing countries.

While at Reading he continued to act as consultant on behalf of organisations of the UN. This work was complementary to his service as Chairman of the University's Overseas Service Committee. Within the UK he was an active member of the Food Group of the Society of Chemical Industry. He was a member of the Food Additives and Contaminants Committee of the Ministry of Agriculture, Fisheries and Food. After retiring from his University post he continued to put great effort into helping with nutrition programmes overseas. He was appointed a member of the UN Advisory Group on Nutrition and was engaged in co-operating with UNEDO in regional services of food industries in the developing countries.

They heavy demands his career made on his time were not allowed to prevent him from playing an active role in the Roman Catholic Church, and recognition was given in 1951 when he was appointed a Knight of St Gergory.

With the untimely death of Francis Aylward, we in food science and technology have lost not only a highly respected colleague, but a friend of great warmth and personal kindness.

E. Rolfe